CEREAL CHEMISTRY

SOFT WINTER WHEAT STUDIES.   III. THE EFFECT OF SOME FACTORS INFLUENCING VISCOSITY AND PROTEIN

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Introduction

The many factors which influence protein quantity and quality have received a great deal of attention by a large number of workers all over the world. A large and ever increasing amount of literature on the subject has resulted. More progress has been made toward solving the effect of various factors upon the *quantity* of protein in wheat than upon the effects produced by these factors upon the quality of the crop. Of the chief factors influencing both quantity and quality of protein, climate is probably the most important, with soil and soil conditions closely approaching climate in importance in an environment such as exists in Ohio. Variety of wheat also produces distinct variations in both quantity and quality of protein. The great difficulty existing in investigations concerning these problems lies in the impossibility of completely eliminating all variables excepting the one being studied. All that one can strive to do is to control the environmental factors as much as possible. One way to reduce the effect of environmental influences is to grow one variety on a uniform soil at one location. A series of wheat samples produced under such a set-up has been studied during the past three years by the author. In this series of wheats the principal variable has been that of soil fertility as influenced by varying fertilizer treatments applied to the soil. It appeared that this series might give information regarding the relative importance of quality of protein as compared with the quantity of protein when one variety was grown under various fertilizer treatments. Does the quality of gluten in a given variety vary with environment or are the observed differences in strength due to varying quantities of protein alone? If protein quality

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is a relatively stable characteristic in any one variety, then the value of the protein determination as a measure of quantity becomes of prime importance, provided the varieties within a given geographical area are standardized and of a uniform protein quality. A second series of samples consisting of several varieties was also used in this study for the purpose of making comparisons with the single variety samples.

Recently the writer reported upon progress made in the evolution of a viscosity procedure suitable for testing soft wheat flours (Bayfield, 1933, 1934a). It appeared that flours from this series of wheats should provide good material for testing out some of these viscosity procedures. Directly comparable data for all three years are not available due to the fact that both baking and viscosity procedures have been changed during the period as desired improvements were made. However, data from any one of the three different years are comparable for that single year at least.

Source of Material

All of the samples were grown at Wooster. Studies dealing with a single variety were made on samples grown on the 5-year rotation plots, on Wooster silt loam soil, during the seasons 1931, 1932, and 1933. These series of samples for the three years were obtained from similar fertilizer treatments and were of one variety, Trumbull wheat. The 1931 series, however, being of a preliminary nature was less extensive than those of the other two years. Some studies dealing with several varieties were also made upon wheat produced on Canfield silt loam in 1933. This soil is closely related to the Wooster silt loam. However, these varieties were grown under fertility conditions quite different from those existing in the 5-year rotation plots.

The 5-year rotation samples were grown in a crop rotation originally laid down in 1893; the rotation of crops being corn, oats, wheat, clover, timothy. A variety of fertilizer treatments was applied; the corn, oats, and wheat crops being fertilized. The west half of each plot received 2 tons of ground limestone applied to the corn crop. The fertilizer treatments applied to the wheat crops being reported upon are given in Tables I, II, and IV. Complete details regarding the 5-year rotation have been presented by Thorne (1924).

Analytical Methods

The samples in this study being a portion of those investigated in the wheat improvement program for Ohio (Bayfield, 1932) were subjected to routine analytical methods approved by the A. A. C. C. (1928). As is customary with this material, all data corrected to a uniform moisture basis have been converted to 15% moisture content. Per-

centage crude protein and ash are therefore given on this basis. Baking test formulae and procedures have been varied to some extent between the different seasons. These changes were found necessary as a result of finding that the A. A. C. C. basic procedure proved unsatisfactory for these experimentally milled flours (Bayfield and Shiple, 1933). However, fermentation times and temperatures stipulated for the basic baking procedure were followed throughout. Similarly, the stipulated amounts of yeast and salt were used. A variable absorption proved advisable and changes were made in the amounts of sugar used. Thus the 1931 crop samples were milled and baked using 3.5 g. of sugar per loaf. Later a second sample of flour was milled and baked with 5.0 g. of sugar, which is now the regulation amount used in this work. This amount of sugar was used for all 1932 and 1933 samples.

Viscosity Procedures

For making the viscosity readings, the procedure proposed by the Subcommittee on the Viscosity Test (Bayfield, 1933) was followed. This procedure, it will be recalled, calls for the use of successive 0, 1, 2, 2, 2 cc. increments of normal lactic acid with a reading being obtained on the viscosimeter immediately after each increment is added. However, three different weights of flour were used, as follows:

1. Regular method where 20 g. of 15% moisture flour was used (constant flour method).
2. Sufficient flour was weighed out to equal exactly 2 g. of protein computed on an uncorrected moisture basis.
3. The same weight of flour as in 2 was weighed and then sufficient wheat starch was added to bring all samples to a uniform weight. The lowest protein flour in each series determined the weight used for the entire series. This method was employed on the 1933 samples only, 24.8 g. being the weight used in the case of the 5-year rotation samples and 22.6 g. for the variety samples. No correction was made for differences in moisture between the flour sample and added starch.

The viscosity readings obtained by the use of 20 g. of flour and the addition of 7 cc. of N lactic acid will be frequently referred to as the "quantity factor" throughout this paper. On the other hand, "quality factor" refers to the viscosity readings from 7 cc. of acid and 2 g. of protein without the addition of extra starch. Data obtained on the 1931 crop samples and elsewhere indicated that a preliminary digestion period before determining the viscosity was desirable. A comparison between results obtained with and without preliminary digestion is given in Table I. Undoubtedly some of the "irregular curves" mentioned by Bayfield (1934) and obtained while working with various experimentally milled flours was due to his using a "no time" (no digestion time) method. The procedure used for all 1932 and 1933 samples, therefore, was as follows:

Weigh out desired amount of flour and place this flour on top of 50 cc. of distilled water previously placed in a 150 cc. beaker. Mix mechanically for 15 seconds using a malted milk mixer operating at normal speed. Stop machine and wash off mixer button with a second 50 cc. of distilled water allowing all the rinsings to fall into the beaker containing the mixed flour and water. During the mixing it has been found advisable to aid the process by rotating the beaker slowly and through the use of a glass stirring rod. The stirring rod, after removal of the beaker from the mixer, is used to stir the suspension a few times, then, after addition of 2 to 4 drops of caprylic alcohol, for stirring a time or two more to further reduce the foam. The beaker is then placed in a water-bath for 1 hour. The water used for making the suspension and also that of the bath should be held at 30° C. It is desirable to hold the laboratory temperatures as close to this temperature as possible during the test. While in the water-bath the suspensions were stirred only once, and this 30 minutes after mixing. It is necessary to stir the suspension again while preparing to pour it into the viscosimeter bowl.

Upon completion of the chemical laboratory phases of the project the data resulting therefrom were subjected to correlation analysis in order to study certain relationships which were under investigation. By this method it was hoped to bring out and separate the *quality* from the *quantity* factor which enters into and complicates any protein study.

Experimental Results and Discussion

1931 Crop Samples

Table I records the data obtained with the 1931 crop samples. These data, excepting the non-bromated bakes, were obtained from a second milling of these samples. However the two millings gave flours of similar protein contents although some variations in ash contents existed (Bayfield, 1933b). The samples in this table have been arranged in order of their protein contents. It will be observed that the viscosity and loaf volumes tend to increase as the protein content in the sample increases. Comparison of the no time (no digestion time) procedure with the digestion method indicates that the same type of a relationship exists in so far as maximum viscosity is concerned. However, with the no time method stable readings were not obtainable in many of the intermediate readings, particularly in the case of readings taken after adding 1 cc. of lactic acid.

Among other points, it was hoped that this study would throw some light upon the possible influence of fertilizer treatment upon protein quality. Percentage flour protein is strongly and positively correlated with the protein in the wheat from which the flour is milled. Studying the influence of fertilizers upon quality of wheat, using percentage protein in wheat and test weight per bushel as criteria, Bayfield (1933a) concluded from a study of 11 years of data from the 5-year rotation that:

1. Phosphatic fertilizers tended to reduce the percentage crude protein and increase the test weight.

TABLE I

DATA FROM 5-YEAR ROTATION, CROP OF 1931

Serial number	1290	1285	1280	1288	1289	1287	1284	1282	1283	1286
Soil treatment [1]	½N2PK	PK	P	½NPK	BYM	NPK	NP	check	N	NK
Plot number	21 E	8 E	2 E	17 E	18 E	11 E	6 E	4 E	5 E	9 E
Flour protein—%	8.6	8.6	9.0	9.4	10.1	10.8	11.6	13.1	13.9	14.3
Flour ash—%	0.48	0.42	0.43	0.43	0.48	0.39	0.41	0.43	0.43	0.43
Loaf volume [2]—Cc.	500	490	510	510	502	505	500	470	500	430
Loaf volume [3]—Cc.	530	555	623	595	653	635	678	700	695	666

Amount N Lactic acid used—Cc.	Viscosity in ° MacMichael—No time method, 20 g. flour									
0	12	13	13	16	16	16	16	19	19	22
1	27	25 [4]	31	22 [4]	22 [4]	25 [4]	34 [4]	64 [4]	30 [4]	46 [4]
3	23	48	44	55	56	68	102	129	136	154
5	41	61	62	73	74	89	120	146	158	176
7	52	68	68	82	81	99	125	151	166	181
9	58	69	68	83	85	103	125	150	166	182

Acid used—Cc.	Viscosity in ° MacMichael—1 hour digestion, 20 g. flour									
0	10	9	10	12	12	13	12	13	16	16
1	16	33	36	77	33	47	70	92	80	96
3	48	73	73	102	82	116	142	175	196	211
5	63	83	81	106	96	129	162	193	213	234
7	69	85	84	107	98	135	162	195	217	236
9	70	86	84	106	98	131	161	193	215	234

[1] N = 160 pounds nitrate of soda; P = 128 pounds 20% superphosphate; K = 80 pounds muriate of potash applied to wheat; 1 = 2 tons ground limestone on corn; BYM = 8 tons barnyard manure on wheat; check = no fertilizers applied since 1893.
[2] Loaf volumes by modified basic procedure, wheat milled Jan. 1932.
[3] Loaf volumes by modified basic procedure + .001 g. potassium bromate, wheat milled March 1933 and flour used for viscosity tests.
[4] Reading point was unstable and result unreliable.

2. Potassic fertilizers produced much less marked but similar tendencies as the phosphates.

3. Nitrogenous fertilizers acted inversely to the phosphates, *i.e.*, increased the protein content and decreased the test weight.

4. Barnyard manure produced the heaviest test weights of all treatments.

5. Lime in *most* years produced heavier yields per acre, higher protein and lower test weight wheat than when the land was not limed.

6. The results indicated that a well balanced nutrient supply was essential for high quality wheat. Of the various fertilizer elements, when applied alone, nitrogen produced the greatest disturbance to the balance in the soil nutrient supply.

The 1933 5-year rotation data presented in this paper is a continuation of the study just cited. The 1933 data are unusual in that it is one

of the few years in which the normal effect of lime was not obtained, *i.e.*, in 1933 the unlimed plots gave higher percentages wheat protein and higher test weight than did the limed plots. Usually the limed halves of the plots give higher percentages of wheat protein and lower test weights than the unlimed ends of these plots.

1932 Crop Samples

Tables II and III give the data obtained on the 1932 crop samples. Both limed and unlimed plot samples were available. It will be observed that viscosity (20 g. flour) and loaf volume tends to increase as the flour protein increases. Non-bromated baking data alone are available but from tests made upon other samples grown at Wooster in 1932 it may be stated that the protein-loaf volume relationship would

TABLE II

DATA FROM THE 5-YEAR ROTATION, CROP OF 1932

Serial number	Soil treatment[1]	Wheat protein	Flour protein	Flour ash	Loaf volume	Viscosity 2 g. protein		Viscosity 20 g. flour	
						1 cc. acid	7 cc. acid	1 cc. acid	7 cc. acid
		%	%	%	Cc.	° MacM.	° MacM.	° MacM.	° MacM.
1653	½N2P½K	9.3	8.2	0.41	500	49	119	38	74
1652	½N2PK	9.6	8.3	.42	550	48	112	38	76
1665	½N2P½K, l	10.0	8.7	.44	530	46	113	40	84
1650	½N2K	9.9	8.8	.42	510	61	131	51	101
1642	P	10.4	9.0	.41	525	55	113	47	90
1664	½N2PK, l	10.3	9.1	.44	565	47	111	42	87
1662	½NPK, l	10.4	9.1	.41	575	56	122	49	101
1654	P, l	10.5	9.2	.42	567	55	115	52	97
1659	PK, l	10.5	9.2	.41	567	58	123	53	105
1647	PK	10.7	9.4	.46	542	34	99	33	88
1661	NPK, l	10.7	9.4	.43	575	60	129	53	110
1646	NP	11.1	9.4	.41	560	53	124	48	108
1649	NPK	11.0	9.7	.40	545	59	125	59	120
1658	NP, l	11.3	9.9	.41	605	51	103	52	103
1663	BYM, l	11.3	10.1	.45	570	49	108	47	108
1651	BYM	11.3	10.1	.42	550	52	110	54	114
1644	Check	12.0	10.9	.42	610	55	104	61	121
1660	NK, l	12.5	11.2	.41	627	61	118	71	147
1643	K	12.7	11.5	.42	608	56	110	67	143
1648	NK	12.9	11.6	.41	665	64	118	77	156
1655	K, l	13.3	12.0	.49	602	48	99	60	144
1656	Check, l	13.3	12.0	.47	605	46	95	55	135
1645	N	13.4	12.3	.41	640	—	—	69[2]	164[2]
1657	N, l	13.8	12.6	.49	570	47	97	64	156

[1] See footnote 1, Table I.
[2] Single determination only, flour supply exhausted.

TABLE III

CORRELATION STUDY, 1932 CROP[1]
N = 23

Total correlation r between	y Flour ash	y Quality[2] factor	y Quantity[3] factor	y Loaf volume	y Flour protein
x	r	r	r	r	r
Flour protein—%	+.5249	−.5596	+.9393	+.7320	—
Loaf volume, regular—Cc.	+.0951	−.2822	+.7507	—	—
Quantity factor—° MacM.[3]	+.3040	−.2537	—	—	—
Quality factor—° MacM.[2]	−.6831	—	—	—	—
Viscosity, 1 cc., 20 g. flour—° MacM.	−.0342	—	—	—	+.0310
Viscosity, 1 cc., 2 g. protein—° MacM.	−.6139	—	—	—	+.0461

Correlation between		Ash held constant	Protein held constant	Quantity[3] factor constant	Quality[2] factor constant
x y	r	R	R	R	R
Loaf volume × flour protein	+.7320	+.8050	—	+.1186	+.7220
" " × quantity factor[3]	+.7507	+.7611	+.2699	—	—
" " × quality factor[2]	−.2822	−.2988	—	—	—
Quantity × quality factor	−.2537	−.0661	—	—	—
Flour protein × quality factor	−.5596	−.3234	—	—	—

[1] Coefficients less than approximately .365 lack statistical significance.
[2] Viscosity in ° MacMichael determined on 2 g. protein samples and 7 cc. N lactic acid.
[3] Viscosity in ° MacMichael determined on 20 g. flour and 7 cc. N lactic acid.

have been still higher had these samples been subjected to the bromate baking test. Unfortunately insufficient flour was available to complete the viscosity tests on sample 1645, one of the most interesting samples in the series due to the wheat having been produced on an unbalanced supply of soil nutrients. Examination of the 2 g. protein viscosity data (quality factor) seems to indicate a reduction in viscosity (quality) with increasing protein content. Even when the ash content is held constant by the partial correlation method this relation is still represented by the coefficient $R = -.3234 \pm .126$. This partial coefficient, however, is not significant if one considers a coefficient as lacking significance when it is less than three times as great as its probable error. The total correlation r between flour protein and the quality factor (viscosity, 2 g. protein) is significant and equals $-.5597 \pm .0966$. Quantity of ash in the flour is seen to be influencing the magnitudes of the viscosity readings. This should be expected. Table III gives certain other statistics obtained during the statistical study of the 1932 crop data.

The principal points brought out by Tables II and III are the outstanding influence of *quantity* of protein and the relatively small influence that *quality* of protein produced. In these samples, flour ash

produced considerable variation and, as might be expected, the ash had a depressing action on the viscosity readings. The relatively small importance of the quality factor in these samples is about that which should be expected seeing that only one variety is concerned in the samples. That different varieties when grouped together produce different results will be shown from the 1933 studies.

Single Variety from 5-year Rotation

1933 Crop Samples

Data for the 24 samples in this group are presented in Table IV. Results from the statistical treatment may be found in Tables V and VI.

TABLE IV

DATA FROM THE 5-YEAR ROTATION, CROP OF 1933

Serial number	Soil treatment[1]	Wheat protein	Flour protein	Flour ash	Loaf volume (regular)	Loaf volume (bromate)	Viscosity 2 g. protein + starch		Viscosity 2 g. protein, no starch		Viscosity 20 g. flour	
							1 cc. acid	7 cc. acid	1 cc. acid	7 cc. acid	1 cc. acid	7 cc. acid
		%	%	%	Cc.	Cc.	°MacM.	°MacM.	°MacM.	°MacM.	°MacM.	°MacM.
2227	½N2PK	9.4	7.9	0.43	540	515	42	99	42	99	34	65
2228	½N2P½K	9.8	8.1	.44	545	520	27	78	38	89	33	65
2215	½N2PK, l	10.1	8.2	.42	555	535	30	87	38	96	34	70
2222	PK	9.9	8.2	.39	560	530	40	98	49	107	41	79
2216	½N2P½K, l	10.2	8.3	.42	557	525	34	88	46	98	41	75
2226	BYM	10.4	8.7	.43	550	520	32	88	50	106	45	89
2210	PK, l	9.8	8.7	.43	595	540	29	88	46	105	43	85
2209	NP, l	10.3	8.8	.44	595	575	27	81	43	99	40	81
2213	½NPK, l	10.4	8.8	.43	550	530	26	81	46	104	43	85
2207	Check, l	10.3	8.9	.34	610	615	86	136	93	142	81	117
2225	½NPK	10.1	9.0	.45	570	550	19	68	37	87	35	74
2221	NP	10.7	9.0	.41	540	530	26	77	44	97	41	84
2212	NPK, l	10.8	9.2	.44	552	550	15	63	28	82	29	73
2217	P	10.7	9.2	.42	565	550	38	92	56	100	52	95
2214	BYM, l	10.9	9.3	.41	585	550	36	96	61	117	58	104
2206	K, l	10.6	9.5	.43	590	555	30	81	52	102	49	94
2205	P, l	11.1	10.0	.45	600	620	36	87	57	108	58	109
2224	NPK	12.1	10.4	.38	620	575	43	93	65	118	67	125
2218	K	12.2	10.4	.37	630	650	64	113	80	125	81	134
2211	NK, l	11.7	10.5	.42	620	655	45	97	65	115	65	122
2208	N, l	11.7	10.5	.39	620	610	52	100	69	114	71	121
2219	Check	12.1	10.5	.39	610	650	58	108	72	118	73	125
2220	N	12.9	11.3	.42	590	620	43	93	60	106	64	128
2223	NK	13.6	11.5	.39	610	650	52	108	75	126	79	151

[1] N = 160 pounds nitrate of soda; P = 128 pounds 20% superphosphate; K = 80 pounds muriate of potash applied on wheat; l = 2 tons ground limestone on corn; BYM = 8 tons barnyard manure on wheat; check = no fertilizer applied since 1893.

As in the case of the 1932 samples all of the intermediate viscosity readings have not been given owing to their bulky nature. The viscosity work on the 1933 samples included one additional new method, *i.e.*, where wheat starch was added in sufficient quantity so that a constant bulk of sample as well as a constant amount of protein was used. The unexpected results obtained from the use of this starch indicated that possibly the starch was introducing a new and unwanted variable. Since working up the data the author has been able to trace the starch to its original source and found the sample to be a " thin boiling " starch,

TABLE V

CORRELATION STUDY, 5-YEAR ROTATION, 1933 [1]

N = 24

Total correlation r between	y Flour ash	y Quality[2] factor	y Quantity[3] factor	y Viscosity, 2 g. protein plus starch	y Loaf volume regular	y Loaf volume bromate	y Flour protein	y Ash in flour plus starch
x	r	r	r	r	r	r	r	r
Flour protein—%	−.3292	+.5041	+.9170	+.3142	+.7528	+.8543	—	—
Loaf volume, bromate—*Cc.*	−.4645	+.6585	+.8844	+.5680	—	—	—	—
Loaf volume, regular—*Cc.*	−.5288	+.7204	+.8417	+.5710	—	—	—	—
Viscosity, 7 cc., 2 g. protein + starch —°*MacM.*	—	—	—	—	—	—	—	−.7932
Quantity factor[3]—°*MacM.*	−.6119	+.8009	—	+.6351	—	—	—	—
Quality factor[2]—°*MacM.*	−.8311	—	—	+.9205	—	—	—	—
Viscosity, 1 cc., 20 g. flour—°*MacM*	−.7511	—	—	—	—	—	+.7825	—
Viscosity, 1 cc., 2 g. protein—°*MacM.*	− 8231	—	—	—	—	—	+.6254	—
Viscosity, 1 cc., 2 g. protein + starch —°*MacM.*	−.8672	—	—	—	—	—	+.4096	−.8487
Wheat protein—%	—	—	—	—	—	—	+.9650	—
Flour yield—%	+.1897	—	—	—	—	—	—	—

[1] Coefficients less than approximately .358 lack statistical significance.
[2] Viscosity in ° MacM. determined on 2 g. protein samples (no starch added) and 7 cc. N lactic acid.
[3] Viscosity in ° MacM. determined on 20 g. flour and 7 cc. N lactic acid.

and therefore not suited to the purpose for which it was used. Accordingly, the data have not been stressed. However, provided a purer sample of starch was used, it is believed that the method of using a constant weight of sample has some advantages over the method whereby the weight of protein alone is kept uniform.

Both analytical and statistical data obtained on these samples indicate the preponderant influence of protein quantity as compared with the quality factor. The bromate baking procedure produced higher correlations with flour protein and with the quantity factor than did the non-bromate procedure. Percentage flour protein in the 1933 crop is

seen to be positively correlated with quality. This may mean that dilution of a given protein quality by starch during the growing period may produce some changes in the protein aggregate so that its strength is reduced. These quality differences may, however, be due to variation in the salt composition of the plant sap, these variations in turn being

TABLE VI

PARTIAL CORRELATIONS COMPUTED FOR THE 1933 SAMPLES

Correlation between x and y with			Factor held constant				
			Flour ash %	Flour protein %	Quantity factor ° MacM.	Quality factor ° MacM.	Viscosity 7 cc. 2 g. protein + starch ° MacM.
x	y	r_{xy}	R	R	R	R	R
5-year Rotation Samples [1]							
Flour protein × loaf volume (bromate)		+.8543	+.8388	—	+.2325	+.8035	+.8649
Loaf volume (bromate) × quantity factor		+.8844	+.8569	+.4872	—	—	—
Quantity factor × quality factor		+.8009	+.6645	—	—	—	—
Loaf volume (bromate) × quality factor		+.6585	+.5075	—	—	—	—
Flour protein × quality factor		+.5041	+.4390	—	—	—	—
Levels of Fertility Samples [2]							
Flour protein × loaf volume (bromate)		+.7107	+.7138	—	+.5666	+.6407	+.6529
Loaf volume (bromate) × quantity factor		+.5219	+.4943	−.0414	—	—	—
Quantity factor × quality factor		+.9373	+.9512	—	—	—	—
Loaf volume × quality factor		+.4030	+.3829	—	—	—	—
Flour protein × quality factor		+.5271	+.4332	—	—	—	—

[1] Coefficients less than approximately .358 lack statitical significance.
[2] Coefficients less than approximately .320 lack statistical significance.

due to the fertilizer treatments applied to the soil. Wood (1907) found a high soluble phosphate content in high quality flours (Fife wheat) and little soluble phosphate and considerable soluble chlorides in weaker flours (Rivett variety). This pioneer work of Wood was later followed by further publications by Wood and co-workers, and others. Bailey (1925) gives a good review of the literature.

Recently Gericke (1933) has shown that wheat grown in aqueous media produced flours with varying protein quantities and qualities. Later he (Gericke, 1934) indicated that the various nitrogen carriers produced wheat, the flours of which gave distinctly different qualities of bread. Of the cations in these fertilizers, calcium produced the best quality, while the ammonium ion gave the poorest results. Gericke lists the five nitrogen carriers which he used as follows: $Ca(NO_3)_2$, KNO_3, $NaNO_3$, $Mg(NO_3)_2$, and NH_4NO_3 in order of decreasing beneficial effect upon bread quality. Gericke believes that the quality of protein is influenced by the cations in the plant sap surrounding the protein aggregate. That different cations should produce changes in the protein complex is not surprising in view of the well known fact that colloids are readily influenced by the presence of various substances in the dispersion medium or intermicellar liquid.

Considering that Gericke was able to obtain changes in bread quality by varying the nutrients supplied to the wheat plant growing in aqueous media, it seems reasonable to expect that varying fertilizer treatments when applied to the soil would also produce changes in protein quality due to the resulting variations in the composition of the plant sap. Ames (1910) and co-workers (1912, 1917), Forbes and co-workers (1913), and Hunt (1927), among others, have published data giving chemical analysis of the ash obtained from wheat grown on various 5-year rotation plots. These analyses indicate that applications of phosphorus to Wooster silt loam soil increases the phosphate content of the grain. Similar results were obtained from a heavy clay soil (Ames and Boltz, 1917). Liming these naturally acid soils increases the amount of phosphorus assimilated by the plant in addition to increasing the amount of calcium and magnesium in the wheat ash. The fact that protein content also increases with liming makes the separation of the quantity from quality factors difficult. In the present study the further difficulty presents itself in that the limed plots did not produce a normal reaction in 1933. In view of these climatic effects it is questionable whether the rather small differences in protein quality expected within one variety can be determined with certainty at this time. The quantity factor as affected by fertilizers is more readily seen. Applications of nitrogen increases whereas phosphorus decreases the quantity of protein produced. Potash produces much less effect than either nitrogen or phosphorus.

Several Varieties from Levels of Fertility Experiment

The principal object in adding these samples to this investigation was to have material consisting of several varieties of varying quality for comparison with the 5-year rotation samples which consisted of a

single variety only. Table VII gives analytical and other data for these varieties. At this time no attention will be given to the reaction of the different varieties to the different levels of fertility. This question will be considered in a later paper since several crops have now been grown previous to 1933.

TABLE VII

DATA ON SAMPLES FROM LEVELS OF FERTILITY EXPERIMENT, CROP OF 1933

Serial number	Variety and level [1]	Wheat protein	Flour protein	Flour ash	Loaf volume (regular)	Loaf volume (bromate)	Viscosity 2 gs. protein + starch		Viscosity 2 gs. protein, no starch		Viscosity 20 gs. flour	
							1 cc. acid	7 cc. acid	1 cc. acid	7 cc. acid	1 cc. acid	7 cc acid
		%	%	%	Cc.	Cc.	°MacM.	°MacM.	°MacM.	°MacM.	°MacM.	°MacM.
2163	Trumbull A	12.4	11.0	0.41	620	705	55	101	66	106	69	118
2164	" B	10.5	9.4	.44	565	590	30	84	49	103	47	87
2165	" C	10.8	9.1	.41	570	555	27	78	39	92	36	72
2166	" D	12.0	10.5	.46	625	600	19	70	43	90	44	92
2167	Fulhio A	10.8	9.5	.46	600	535	16	73	37	91	36	79
2168	" B	10.8	9.5	.45	572	565	26	84	48	104	45	90
2169	" C	10.8	9.2	.39	567	555	46	97	60	113	53	92
2170	" D	11.8	10.2	.43	595	577	26	71	46	91	46	89
2171	Gladden A	11.8	10.3	.43	630	655	45	82	51	86	49	85
2172	" B	10.5	8.9	.41	572	550	43	84	50	89	43	70
2173	" C	10.3	8.8	.46	550	540	27	67	33	74	31	59
2174	" D	10.9	9.1	.40	585	535	52	89	58	96	49	76
2175	Michikof A	13.4	12.4	.47	645	670	59	136	80	162	93	217
2176	" B	12.5	10.8	.45	622	597	63	149	95	182	90	200
2177	" C	12.2	10.8	.48	560	530	44	123	80	163	90	181
2178	" D	12.8	11.9	.50	630	620	29	101	72	151	87	197
2179	No. 6 Jr. A	12.0	10.8	.49	557	582	20	49	30	59	29	61
2180	" B	10.4	8.9	.40	530	505	33	62	35	64	29	49
2181	" C	10.5	8.8	.41	497	460	31	60	33	63	27	46
2182	" D	11.3	9.5	.42	500	475	21	46	28	54	25	45
2183	T. N. 1006 A	11.6	10.3	.43	610	640	41	85	50	91	49	89
2184	" B	10.6	9.3	.38	620	590	50	100	54	104	51	88
2185	" C	10.6	9.0	.40	565	540	45	94	53	103	48	84
2186	" D	11.3	9.4	.40	577	535	36	84	49	100	47	86
2187	T. N. 1047 A	12.3	10.6	.43	627	680	47	94	56	100	57	103
2188	" B	10.3	8.9	.43	610	555	56	109	67	121	57	93
2189	" C	10.2	8.6	.41	602	550	61	113	61	113	52	86
2190	" D	11.4	9.4	.42	562	560	41	89	60	107	53	89
2191	Dawson [2] A	12.0	10.1	.51	545	570	19	56	33	74	30	63
2192	" B	10.4	9.1	.39	550	540	42	81	45	87	39	68
2193	" C	10.5	9.0	.48	525	515	16	53	25	62	24	51
2194	" D	11.4	9.6	.42	545	535	30	70	42	84	37	70

[1] 3-year rotation of corn, oats, wheat. Fertilizer treatments—A, none; B, 4 tons manure, 100 lb. 0-16-0 broadcast, 100 lb. 4-12-4 in hill on corn; oats not fertilized; wheat receives 200 lb. 2-14-4 in fall and 50 lb. nitrate in spring; C, twice the amounts of B level; D, twice amounts of the C level.
[2] Dawson mixed with some red wheat.

The eight varieties in this set of samples cover quite a range in strength and quality, ranging from the strong gluten but poor milling variety, Michikof (Bayfield, 1932a), to the weak white winter wheats, Dawson (Dawson's Golden Chaff) and No. 6 Junior. Trumbull and Fulhio, both Ohio selections from Fultz, constitute about 85% of the total Ohio crop. Gladden is a low protein soft red winter which normally possesses uniformly soft textured grains. Nabob, on the other hand, while being inclined to be a low protein variety, exhibits a tendency to be very variable in kernel texture. The two varieties TN 1006 and TN 1047 are new Ohio selections as yet not distributed to the public. These tests indicate that the former of these new varieties compares favorably with the check variety, Trumbull, whereas the latter variety is somewhat strong for Ohio. Gladden is usually considered a weak variety. These tests indicate that its reputation has been earned by its low protein content and not due to any inherent lack of good gluten quality.

While it is not the intention at this time to discuss the relative merits or demerits of various baking procedures, yet the baking tests on these samples are interesting. The baking data from the five-year rotation samples indicate that the use of potassium bromate depressed loaf volume until this Trumbull wheat flour contained in the neighborhood of 10% of protein. Above this figure the use of bromate produced a stimulation and larger loaves resulted than where no bromate was used. Of the several varieties given in Table VII all show increased loaf volumes with bromate when grown on Level A fertility excepting Fulhio (Level A gave the highest amount of protein). With one exception (Trumbull, Level B) all the rest of the samples were depressed by the use of bromate. Swanson and Kroeker (1932) working with hard wheats in Kansas thought that response to bromate was hereditary. These Ohio samples indicate, however, that for these varieties at least, the response to bromate was due to quantity of protein rather than to an inherited quality of protein.

The data from these samples have been subjected to correlation analysis as in the case of the 5-year rotation data. Table VIII gives the total correlations calculated, partial correlations being presented in Table VI.

Comparison of the coefficients obtained on the 5-year samples (Table V) with those given in Table VIII indicates that the relationship between protein content and loaf volume was reduced by the introduction of several varieties having varying degrees of gluten quality. Similarly, the protein percentage x quantity factor relationship was reduced. The quality factor x protein correlation was slightly increased.

Table VI brings together the partial coefficients calculated from the

TABLE VIII

CORRELATION STUDY, SEVERAL WHEAT VARIETIES, 1933 CROP [1]

N = 32

Total correlation r between	y Flour ash	y Quality factor [2]	y Quantity factor [3]	y Viscosity 7 cc. 2 gm. protein + starch	y Loaf volume regular	y Loaf volume bromate	y Flour protein
x	r	r	r	r	r	r	r
Flour protein—%	+.0594	+.5271	+.7609	+.3853	+.5890	+.7107	—
Loaf volume, bromate—Cc.	+.1928	+.4030	+.5219	+.4423	—	—	—
Loaf volume, regular—Cc.	+.0913	+.6185	+.6394	+.6591	—	—	—
Quantity factor[3] —°MacM.	+.3516	+.9373	+.8330	—	—	—	—
Quality factor[2] —°MacM.	+.1702	—	—	+.9422	—	—	—
Viscosity 1 cc. 20 g. flour—°MacM.	+.1831	—	—	—	—	—	+.6639
Viscosity 2 g. protein—°MacM.	+.0060	—	—	—	—	—	+.4906
Viscosity 2 g. protein plus starch—°MacM.	−.4193	—	—	—	—	—	+.1192
Wheat protein—%	—	—	—	—	—	—	+.9685

[1] Correlation coefficients less than approximately .320 lack statistical significance.
[2] Viscosity in ° MacMichael from 2 g. protein and 7 cc. N lactic acid.
[3] Viscosity in ° MacMichael from 20 g. flour and 7 cc. N lactic acid.

data of the two 1933 series of samples. In this table percentage flour ash is the principal factor which was held contant. Normally it would be expected that the elimination of ash as a variable affecting the viscosity results would improve these viscosity relationships. The conflicting results obtained in this case are probably due to the fact that the protein-ash relationship in these flours was frequently abnormal due to the fact that the flours were experimentally milled and thus less control was possible than would have been the case had large samples been commercially milled. Usually, a positive correlation exists between protein and ash in flour, but on these 1933 samples no significant relationship obtained. In fact the 5-year rotation samples showed a negative tendency, i.e., for protein to increase as the ash content went down. The table, however, does show something about the protein. Thus protein quantity is the most important factor in the protein content of these samples in so far as strength is concerned. With a single variety the quality factor is much less important than where several varieties are being grouped together. This fact is important because much of our wheat today is purchased on a protein basis and many wheat blends are blended on a protein basis. The protein determination is a measure of quantity, and not quality. Therefore the determination itself loses more and more value as the wheat on the market lacks standardization as to quality, and variety of wheat is a very important factor in producing these variations in quality.

Summary

By means of viscosimetric methods an attempt was made to separate the quality from the quantity factors existing in the protein content of flours.

Quantity of protein was readily measured and these viscosity results were found to be well correlated with results from baking tests.

Quality of protein was found to produce much less influence upon viscosity when only one variety of wheat was used throughout the series. When several varieties were compared, however, decided differences in quality were obtained.

Applications of fertilizers to the soil growing the wheat produced large variations in quantity of protein. While differences in quality were noted these differences were small and the results inconclusive due to the unusual effect of climate during one of the years under study.

The fertilizer elements, nitrogen, potassium, and phosphorus produced decreasing amounts of protein with phosphorus producing the least.

Response to potassium bromate when employed in baking these varieties seemed to be due to quantity rather than quality of protein.

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THE MEASUREMENT OF COLOR IN FLOUR AND BREAD BY MEANS OF MAXWELL DISCS. II

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In a previous publication (1933) the authors described a colorimeter for the measurement of color in flour and bread and reported data obtained with that machine. Further research along similar lines has revealed various errors that were inherent in this colorimeter and as a result a machine has been developed that can be more easily and accurately used to measure the color of bread and flour.

A series of measurements on samples from one flour over a long period of time showed variations greater than those due to errors of observation. The percentage of black was at a maximum in the early morning and gradually decreased to a minimum in the late afternoon. The yellow showed an opposite trend, being at a minimum in the morning and reaching a maximum in the late afternoon. These measurements were confirmed by repeated tests and indicated that the source of light was not uniform throughout the day. This conclusion led to experiments with various types of lamps and as a result a lamp and appropriate set-up was devised to replace daylight as a source of illumination.

Another objectionable feature of daylight as a source of illumination is the constant variation of intensity due to clouds passing in front of the sun. These variations, due to the inability of the eye to adjust itself rapidly to sudden changes, cause eyestrain with resulting errors in judging color matches. In using the daylight lamp similar fluctuations of intensity are caused by line voltage variations. To overcome this difficulty a rheostat is used in the lamp circuit and a constant intensity can be maintained during measurements. The intensity is measured by means of a Weston Photronic cell connected to a micrometer.

While re-designing the colorimeter for artificial illumination several mechanical changes in design were adopted. These are in brief:

(1) Smaller color discs and sample dishes, the new size being 2½ inches in diameter in place of the former 4 inch size. This makes preparation and handling of samples easier and also permits samples of bread and cake to be made from a single slice.
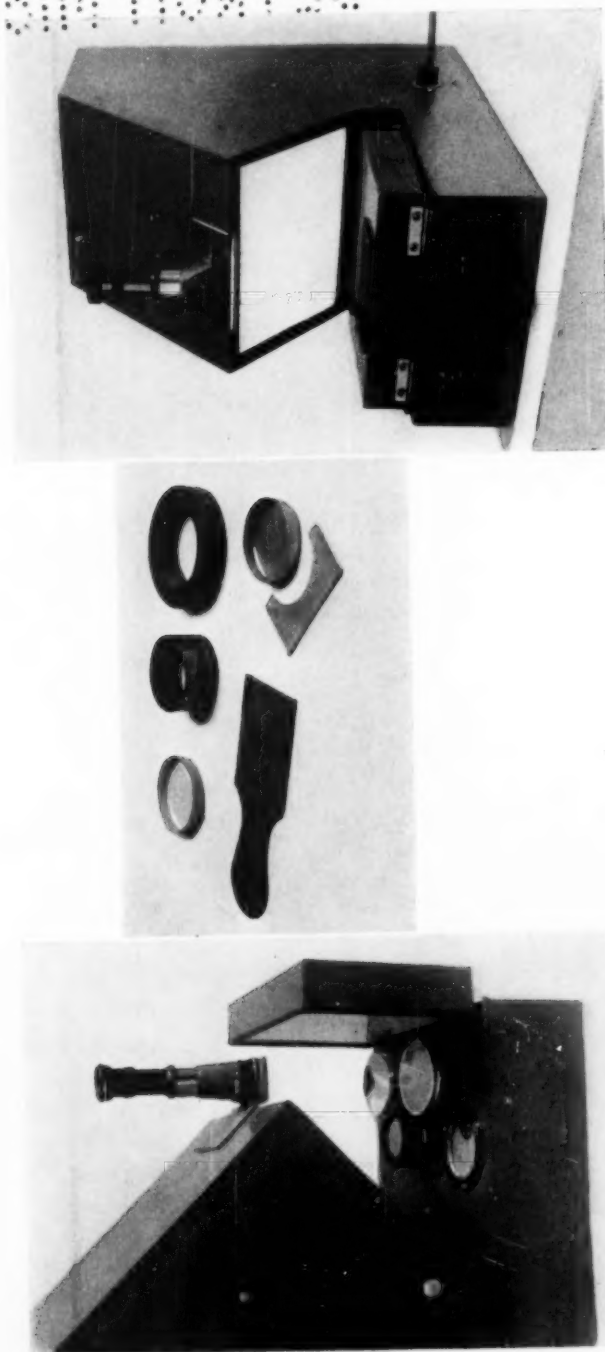(2) The optical comparator is made a permanent part of the apparatus and is used on all readings.

Fig. 1.

(3) The daylight lamp operates at over voltage. In order to protect it from un-
necessary use, which would cause deterioration with consequent change in
light quality, a thrust type switch is used. This switch is depressed by the
finger while the observer is actually looking through the comparator. When
depressed it lights the daylight lamp and starts the color discs spinning.
When the finger is released the switch turns off the daylight lamp and stops
the discs, at the same time lighting an auxiliary lamp. The auxiliary lamp
gives sufficient light to adjust discs, measure color sectors, etc.
(4) A new slicking block, used with a flour slick, eliminating several cumbersome
accessories.

Figure 1 illustrates the new model N–A disc colorimeter embodying
the above features.

In order to determine the accuracy of the colorimeter a series of
twenty (20) readings were taken by a single experienced observer on
the same sample and statistical constants calculated according to Treloar
(1929, 1931) and Treloar and Harris (1928). A comparison of these
constants with similar ones determined for the old type using daylight
is given in Figure 2.

Fig. 2.   Comparative statistical constants.   20 wet slick readings on
untreated H. W. Patent

I. STANDARD DEVIATIONS (6 ± P.E. 6)

	NEW MODEL COLORIMETER USING DAYLIGHT LAMP	OLD MODEL COLORIMETER USING NORTH SKYLIGHT DIFFUSED THROUGH OPAL GLASS
YELLOW	0.1025±0.0109	0.5355±0.0452
RED	0.0866±0.0092	0.3180±0.0268
BLACK	0.1140±0.0122	0.0876±0.0074
WHITE	0.0274±0.0221	0.4999±0.0421

II. COEFFICIENTS OF VARIATION (C.V.)

YELLOW	0.38%	1.70%
RED	1.08%	4.83%
BLACK	6.70%	15.65%
WHITE	0.33%	0.82%

The coefficients of variation given in Figure 2, section II, show that
the new colorimeter is an improved machine from the standpoint of
accuracy, when compared with the first model.

Previous studies of flour and bread color (Baker *et al.*, 1933) showed
a surprising amount of black in bread as compared with flour. This
was assumed to be due to its translucent character and to shadow effects
in the crumb. At that time samples of bread were pressed in a hy-

draulic press and the increase in the black characteristic noted, but no careful study was made of this type of sample.

In the present study a technique was worked out to make samples of pressed bread crumb in which texture marks, bubbles, etc., were completely eliminated. This consisted of pressing the bread crumb between two large, flat plates in a hydraulic press. The maximum pressure used to press the samples was approximately 3000 pounds per square inch of sample. The crumb under this tremendous pressure becomes a very thin sheet approaching transparency. After each pressing the sheet is rolled into a ball and again subjected to pressure. This procedure is followed until no texture marks are present and the crumb is a thin, uniform, semi-transparent sheet. The sheet is then cut into circular pieces $2\frac{1}{2}$ inches in diameter. The pieces are piled on top of each other to form a cylinder 2 inches high. As each piece is added to the pile it is rolled carefully into place to prevent any entrapped air. The 2-inch high cylinder is again placed between the plates, using 1-inch " stops," and pressed to the height of 1 inch. This pressed mass is cut into a cylinder $2\frac{1}{2}$ inches in diameter and 1 inch high. The resulting sample is a uniform mass of bread substance. The above procedure represents a compression to approximately $\frac{1}{6}$ the original thickness of the bread crumb.

In the case of steam cooked dough the original mass is more uniform and requires fewer pressings. The resulting sample is again a uniform cylindrical mass $2\frac{1}{2}$ inches in diameter by 1 inch in height. In this case, however, the actual compression of volume is slight, the process being used only as a means of working out the small amount of air present.

The pressed sample appeared more translucent than any other type of sample previously studied and hence it was deemed advisable to determine its translucency and also that of the more common types of samples. This was done by placing standard color discs under a thick sample, reading the color, and then gradually decreasing the thickness until the underlying color affected the reading of the colorimeter. The thickness through which light penetrated was determined using each of the four standard color discs, *i.e.*, yellow, red, black, and white. It was found that for a given type of sample the translucency was substantially independent of the underlying color.

Figure 3 shows the results obtained using the above procedure.

It will be noted that wetting flour to produce a dough increases translucency 8 times, and that when dough is steam cooked this increase in translucency is tripled.

Light also penetrates a layer of bread crumb 40 times as thick as dry flour and penetrates a still deeper layer of pressed crumb.

Fig. 3. Study of translucency
(Untreated Hard Wheat Short Patent)

	SAMPLES THAT ARE EQUALLY TRANSLUCENT		
	THICKNESS (in 1/64")	WEIGHT (grams)	WT. OF FLOUR IN SAMPLE (grams)
DRY FLOUR	1	1	1
DOUGH (FLOUR & WATER)	8	13	8
STEAM COOKED DOUGH	24	35	24
BREAD CRUMB	40	10	8
* PRESSED BREAD CRUMB	56	82	61

* Pressed steam cooked dough gives results closely approximating these figures.

It is also evident from Figure 3 that though a thick layer of bread crumb is penetrated by light, this represents only a comparatively small weight of flour. That this difference is due to texture is demonstrated by the fact that when the texture is completely eliminated by pressure in a hydraulic press the weight of flour penetrated increases almost 8 times. It should also be noted that steam cooked dough is not entirely uniform. Hence, when the bubbles and irregularities are removed by pressing the penetration is increased to a figure closely approximating pressed bread crumb.

The effect of grade of flour on the color of pressed bread crumb is shown in Figure 4.

Here a definite color difference exists between grades, the yellow and white decreasing and the red and black increasing with lowering of grade.

Fig. 4. Pressed bread crumb. Variation of color with grade.
(Hard Wheat Flour)

	UNTREATED SHORT PATENT	UNTREATED FIRST CLEAR	UNTREATED LOW GRADE
% YELLOW	22.0	18.0	12.0
% RED	6.8	9.1	8.0
% BLACK	52.2	58.7	70.0
% WHITE	19.0	14.2	10.0

These studies show that the deeper the light penetrates the greater the absorption by the impurities and pigments present and the less light is reflected. Since black is absence of light, this decreased reflection, due to penetration with consequent absorption, means an increase of black. Anything that tends to increase the reflecting surface such as bubbles, texture, granulation, etc., decreases penetration, causes a decrease of black and the sample appears whiter. This explains why bread with a fine texture is whiter than a similar loaf with a coarse texture. It also explains why a finely ground flour is whiter than a granular flour milled from the same wheat.

Samples of the same flour in different forms were analysed for color. These readings are given in Figure 5.

Fig. 5. Variation of color with type of sample
(Untreated Hard Wheat Short Patent)

	DRY FLOUR	WET SLICK	STEAM COOKED WET SLICK	DOUGH (FLOUR AND WATER)	BREAD CRUMB	STEAM COOKED DOUGH	PRESSED BREAD CRUMB	PRESSED STEAM COOKED DOUGH
% YELLOW	14.9	30.2	38.7	26.5	24.5	28.0	22.0	19.0
% RED	1.9	7.5	8.3	9.3	7.0	11.0	6.8	7.0
% BLACK	0.0	1.9	9.0	17.2	22.8	32.4	52.2	56.0
% WHITE	83.2	60.3	44.0	47.0	45.7	28.6	19.0	18.0

The samples are arranged in order of increasing percentage of black, varying from 0.0% in the case of the dry flour to 56.0% in the pressed, steam-cooked dough. These results show that high black readings are not primarily caused by shadows in the texture, the highest readings being obtained on a uniform mass.

Since wetting, heat and pressure affect translucency, we can explain the readings in Figure 5 on the basis of this same theory.[1]   In the case of dry flour, the sample is not very translucent, most of the light striking the many surfaces is reflected and we have, in this exceptionally white flour, 0.0%[2] black. When the dry flour is dipped in water to make a wet slick, a translucent film is formed and light penetrates deeper into the sample. Some of the light is reflected and some absorbed by the film, the remainder is reflected from the unwetted flour underneath with

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[1] This theory does not include the changes taking place during the drying of a wet slick or bread crumb. The increase of black in these cases is probably caused by oxidation and enzymatic action.
[2] This does not imply that the sample is a perfect white, but rather that it is as free from black as the standard color discs.

more absorption as it passes again through the film and as a result the black increases. When this wet slick is cooked the film becomes more translucent and more light penetrates. This leads to more absorption with a consequent increase of black.

Alcock and Ediger (1929) studied moisture content of flour as affecting the color obtained using the Pekar test. They concluded that the thickness of film was determined by the moisture content of the flour sample and that the color observed on the slick was greatly influenced by the thickness and opacity of this film. · Our observations support this conclusion.

In the case of a dough made of flour and water, all the particles are wet and the mass is more translucent. Light penetrates deeper into this sample than in the slick and having no highly reflective, dry, granular flour under the surface does not appear as white as does a wet slick. When dough is cooked the penetration of light is further increased and the mass appears darker. In pressing cooked dough in a hydraulic press all irregularities, bubbles, etc., are removed. As pointed out these bubbles help reflection and hence their removal causes an increase in translucency and an increase in black.

Bread having a texture consisting of innumerable bubbles is a better reflector than the cooked dough and hence has less black. In pressing, however, these texture effects are eliminated and the result is a marked increase in black due to greater translucency.

As light penetrates deeper into the sample, the color characteristics of the sample are shown increasingly in the quality of the reflected light. This is manifest by the increase in yellow and red in the successive samples in Figure 5. However, when the absorption of light by the sample becomes unduly great, these colors are also absorbed and their intensity reduced. This is apparent in the falling off of these colors in the pressed samples. Their decrease accounts for part of the marked increase in black.

Jago (1921) examined and compared doughs from a color standpoint, and suggested the use of dough color as an indication of bread color. His work shows that dough color is a more reliable method for this purpose than either the dry flour or wet slick.

Rüter (1931) in a review of flour colorimetry also recommends the use of a small dough as the best method of judging bread color.

From Figure 3 it is evident that approximately the same weight of flour is penetrated in dough as in bread crumb. This fact probably explains the color similarity noted by Jago and Rüter. Also of the various readings in Figure 5 dough color seems to be nearer to bread color than any other type of sample. Thus dough color apparently is the best

method of judging the bread color that will be obtained from a given flour.

Further studies of this relationship are being made by the authors.

Summary

The new N–A colorimeter described in this paper has many advantages over the old type. Preparation of samples is facilitated and greater ease of operation permits more rapid and less fatiguing observation. The use of standard artificial illumination leads to greater accuracy because readings are taken under constant quality and intensity of light.

Studies of flour and bread color point to translucency of a given sample as the most important factor governing the color obtained in the various physical states in which it is measured. That is, the color differences observed between dry flour, dough, bread, etc., are due largely to differences in light penetration producd by wetting, heating, texture and granulation.

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AN INDEX OF PROTEOLYTIC ACTIVITY BY THE USE OF THE FARINOGRAPH

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The Farinograph (Simons, 1932) has been generally applied to the testing of inherent flour qualities such as gluten strength (Lange, 1933), but, as far as is known, a quantitative interpretation of enzyme effects as shown by this instrument has not been entirely successful (see Mueller, 1933, and Brabender, 1932).

Preliminary tests with the " rest period " method seemed to indicate that the elasticity, *i.e.*, the width of the Farinograph record, was a complex of several factors (Szegfy, 1933). Hence the damping adjustment, previously set at 0.8 second (from 1000° B. to 100° B.) for our 60 cycle current, as recommended by Dr. Brabender, was changed to give the maximum damping of which the machine is capable. Elasticity variations became negligible and the other constants of the curve could be readily obtained. The general shape remained unaffected.

A Standard Substrate

If measurements of this nature are to be accurately comparable, it is necessary that a standard substrate be prepared. It was found that the incorporation of added ingredients at the beginning of mixing, particularly if they were possessed of high activity, introduced considerable variations in the type of mixing received, and in the consistency at the end of mixing, even in a two- or three-minute mix. In order to eliminate these variations the procedure was modified to obtain a standard dough before the addition of the enzyme. The flour was titrated to a consistency of 600 at exactly five minutes after the beginning of mixing. This constituted the standard substrate, a standard dough developed to a reproducible amount, suitable for the addition of other ingredients. Then 10 cc. of water which might also contain enzymic or dough ingredients, was gradually added over a period of one minute, mixing continued for one minute more to re-develop the dough, and the dough removed from the mixer, if desired, and allowed to rest. At the expiration of the desired time it was replaced in the mixer and the test curve obtained. The general shape of the curve is indicated in Figure 1.

This curve may be accurately reproduced at will, although frequent titrations are advisable. It was found necessary to weigh all samples of flour at the same time from a well mixed bin; even then the titration frequently changed as much as 0.5 cc. or more from morning to afternoon. Variations in final consistency, even one minute after completing the addition of a highly active enzyme in the 10 cc. of water, rarely exceeded 10° B.
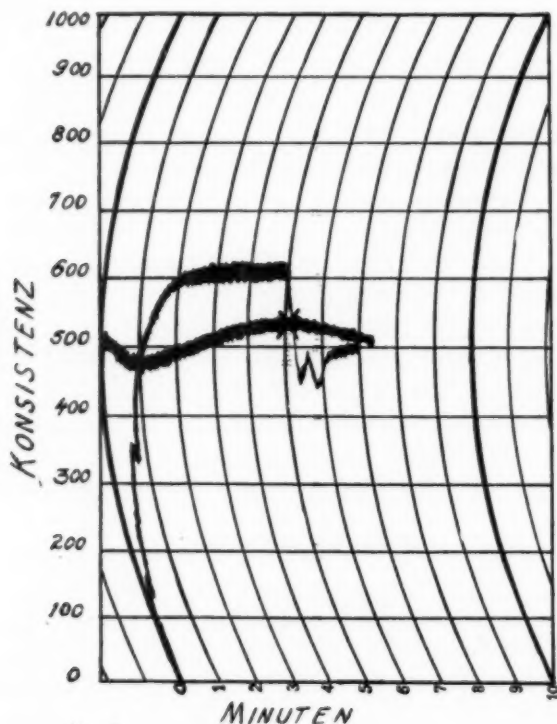

Fig. 1. Typical test curve.

Since the presence of salt has been reported frequently to have a marked influence on enzymic activity, it was thought desirable to include this ingredient in the standard substrate. Although it changes the titration value of the flour (see de Jong, 1933) it has relatively small effect on the characteristics of the substrate curve in the case of strong Northwestern flours. It was also found to increase the period of development, and hence the sensitivity of the test.

For the preparation of the standard substrate it may be pointed out that some flours seem inherently suited for the purpose. For example, a Montana type flour, with a long period of gradual development and a

sharp peak, or a Southwestern type flour are probably not suitable. It is difficult to reproduce titrations with such flours. Some Northwestern patents develop fairly rapidly and have a long flat or nearly horizontal peak or plateau, and the amount of water necessary to produce the standard consistency may be easily and accurately determined. For particular purposes, however, it may be found that flours giving sharp peaks may be quite desirable.

Determination of Time and Concentration Limits

Standard doughs were prepared and varying amounts of papain were added, the rest periods being varied from one to twenty-four hours, and the test curves analyzed (see Table I).

TABLE I

EFFECT OF PAPAIN ON AVERAGE CONSISTENCY OF STANDARD DOUGH

Papain, mg. per 300 g. flour	Time of action, hours	Drop ° B. (from blank test)
5	3	0
10	3	5
50	3	275
5	6	0
10	6	5
50	6	325
5	12	30
10	12	46
50	12	330

It was found that over a critical point, approximately 40 mg. of the sample of papain used per 300 g. of flour, the nature of the curve changed. The consistency dropped suddenly, the period of development diminished to zero, and the curve became characterless. If this point could be determined as a function of time and concentration, it might serve as a measure of the activity of the enzyme. No simple method, other than the accumulation of a large series of curves and interpolation, seems to be available. In order to show the complete character of the curve it was found necessary to mix until the appearance of the peak. This necessitated a 15-minute test mix in many instances, and further decreased the number of determinations which could be made. Hence the early stages of the reaction were investigated. The data are recorded in Table II. The greater inherent capacity and smaller initial enzymic activity of the Northwestern patent as compared with the Southwestern flour may be inferred from this table.

TABLE II

EFFECT OF PAPAIN ON DEVELOPMENT TIME

Flour	Papain, mg. per 300 g. flour	Time of action, hours	Maximum consistency ° B.	Development time, minutes
Northwestern patent	Nil	0	550	14
	Nil	3	490	10
	50	1	455	3.0
	50	2	400	2.2
	50	3	355	1.3
Southwestern patent	Nil	0	540	8.2
	Nil	3	440	6
	50	1	245	1.10
	50	2	170	.75
	50	3	150	0–.1

"Development Energy" as a Measure of Gluten Condition at Any Instant

While in the early stages the drop in consistency is relatively small compared with the decrease in developing time, it cannot be neglected. It is likely that neither factor will serve as an index of the activity, since in certain cases an apparent increase in maximum consistency accompanied by a decrease in developing time have been observed (certain malt extracts). Hence it seems probable that the product of the two, or better, the area under the curve, taking both factors into consideration, might serve as a more suitable index.[1] The area under the test curve up to the point of maximum development is an indication of the amount of energy required to redevelop the test dough to its maximum extent. From theoretical considerations it seems logical that the inherent colloidal characteristics of the gluten should be reflected in an index of this nature. As these are changed by incipient chemical or enzymic action, far too small to be detected by the usual chemical means, marked variations in this characteristic should occur.

Since the record appears on the chart as torque units required to move the mixing blades through the dough at constant speed (power), the product of these units by the time has the dimensions of energy. Expressed in degree Brabender-minutes (°B.-minutes), this product, taken to the time of maximum development (center of the peak or plateau in the test curve), has been tentatively termed the energy of development, or "development energy" of the test dough. Decreases in this energy after action of enzymes may tentatively be taken as a measure of the degradation produced.

[1] According to Dr. Brabender this has previously been studied as an index of flour characteristics. In this case, however, only the area under the test curve is considered, the relatively constant amount of development given in the preparation of the substrate being discarded.

Although small variations in absorption have been found to be negligible in their final effect, nevertheless all measurements herein reported are made at constant initial consistency. Under these conditions remarkably reproducible results may be obtained, as illustrated by Table III.

TABLE III

REPLICABILITY OF SUBSTRATE

Flour	Rest, minutes	Development energy, kilo units [1]
Northwestern patent " A "	0	7.54
	1	7.85
	5	7.74
	1	7.64
	0	7.95
Average		7.74
Standard deviation		.15
Coefficient of variation		1.9%
Northwestern patent " B "	1	7.18
	1	7.13
	1	7.07
Average		7.13
Standard deviation		.05
Coefficient of variation		0.8%

[1] No name has yet been given to the unit of development energy. Data are taken from the curve by the use of a plainimeter, and converted to D. E. units in °B.-minutes. 1000 °B.-minutes— 1 " kilo unit."

Rate of Degradation of Gluten as Measured by the Decrease in Development Energy as a Function of Enzyme Concentration

Using the method described below the change in development energy with time and amount of added papain was determined. Since at the critical point mentioned above the development energy becomes zero, after which only decreases in average consistency become apparent, measurements were limited to conditions under which this value remains finite. The development energy was found to decrease uniformily during the first one-half to three-quarters hour. At one hour, even in the plain dough, an abrupt change in rate of degradation takes place. The initial slopes, however, were found to be roughly proportional to the amount of added enzyme (compare Figure 2).

It has been shown that the initial development energy of the dough is a relatively constant characteristic of the flour, and replicate measurements agree with considerable accuracy. According to our conceptions of the nature of enzyme action and measurement, the initial time rate of decrease of this value should be a direct measure of the proteolytic
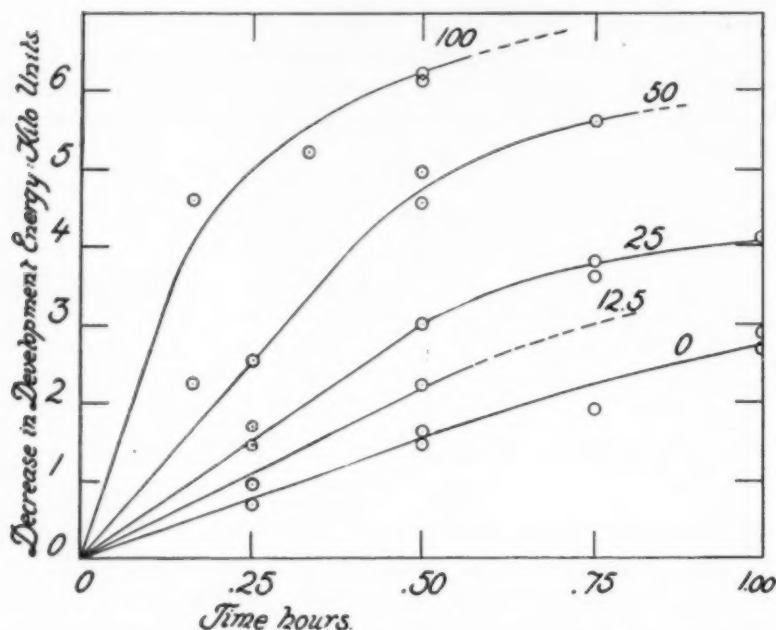
Fig. 2. Decrease in development energy with time and concentration of proteolyst. The figures identifying the curves indicate the number of milligrams of papain per 300 grams of flour.

activity in the dough (Johnston and Jozsa, 1933). This rate of decrease has been found to be nearly constant for one enzyme concentration up to at least one-half hour after addition of the enzyme and for upwards of 60% of the total change, provided this occurs within that time limit. If this rate varies directly as the enzyme concentration, it will serve as a method for estimating the proteolytic enzyme content of commercial preparations. The decreases in development energy for various concentrations of papain are shown in Figure 2. From this plot the initial rates may be evaluated and compared (Table IV).

TABLE IV

EFFECT OF ENZYME CONCENTRATION ON OBSERVED ACTIVITY

Added enzyme, mg. per 300 g. flour	Initial slope, kilo units per hour	Enzyme unit added in papain	Observed proteolytic activity, enzyme units per gram
0	3.0	0	0.01
12½	4.3	1.3	104
25	5.9	2.9	116
50	10.0	7.0	140
100	28.0	25.0	250

Enzyme Unit

It will be observed that the flour itself possesses an enzymic activity, which may be real or apparent. In any event, it is logical to assume that this activity will not be blanketed by any added enzyme, *i.e.,* the latter's activity will be superimposed upon that of the former. Hence, the activities recorded in Table IV are corrected for the apparent activity of the flour itself. This method of procedure also has the advantage that a standard flour need not form the basis of the method. There is apparently a slight tendency for the ratio, slope of the curve to added enzyme, to increase, a tendency which becomes very pronounced when the activity is made greater than that equivalent to 50 mg. of this sample of papain per 300 g. of flour. If, therefore, the rate of decrease development energy be limited to, say for the present not over 10 kilo units per hour, this procedure may be made the basis of a method for proteolytic activity. We may, therefore, define the enzyme unit as follows: One unit of proteolytic enzyme is that amount (a definite but as yet unknown number of active particles) which, added to or present in the standard dough, will cause an *initial* rate of decrease in development energy of one kilo unit (1000 °B.-min.) per hour. As defined at present the standard dough is that prepared in the Farinograph from a patent flour with an initial test dough development energy in the neighborhood of 7 kilo units, moderately rapid mixing development, and no pronounced peak of development, such as a Northwestern patent flour.

Details of Method

Mix the flour thoroughly and weigh out enough samples (300 g.) to last for one series of tests. These samples should be kept in tight tin containers. Prepare a salt solution containing an amount such that the final concentration in the dough will be equivalent to 1% of the flour (16.7 g. per 1000 cc. of water if the normal absorption of the flour is around 60%). Titrate a sample of the flour with this salt solution to a consistency of 600° Brabender, in a farinograph with damping (dash pot) adjusted to its maximum value. The titration may occupy from 4 to 4.5 minutes, but the curve during the last one-half minute should remain essentially constant at 600° B.

Prepare enzyme solutions of appropriate strength. For the determination of the initial value of the flour and its enzyme content plain water may be used. Suitable concentrations have to be found to be 0.1 to 0.2 g. papain per 100 cc., 10 g. of highly diastatic malt extract per 100 cc., 30 g. low diastatic malt extract per 100 cc. If the solids content of the enzyme solution exceeds 1 g. per 100 cc., inactivate a portion of the solution by placing in a boiling water bath for one-half hour and determine the apparent enzymic activity of this inactivated solution along with that of the original sample. This is necessary to correct for any osmotic effect or change in absorption caused by the solutes.

Using the titration value previously determined, prepare a standard dough in the Farinograph, adding all the salt solution as fast as it will flow from the burette. Precisely at the end of five minutes add 10 cc. of the enzyme solution (water in the case of blanks), active or inactivated. The addition should be made slowly, dropwise, taking exactly one minute to complete. This is necessary to insure uniform and rapid incorporation into the rather stiff dough. Mix for an additional minute,

making seven minutes from the beginning of the addition of salt solution. Now remove the dough carefully and rapidly from the mixing chamber and allow to rest at 30° C. for 30 minutes, counting time from the beginning of the addition of the enzyme. Replace the dough in the farinograph and mix until the maximum consistency has been reached and passed.

Remove the chart from the machine and determine the center of the region of maximum development. Determine the area under the test curve up to this point in kilo units. This value, subtracted from the initial development energy of the standard test dough (determined with 10 cc. of water, allowing one minute rest before testing), gives the decrease in development energy. Twice this value is the rate of decrease in development energy in kilo units per hour, and is equal to the number of enzyme units in the dough by definition. Subtract from this value the number of enzyme units added with the flour (determined by blank test), correct for the apparent enzymic activity of the solids accompanying the enzyme concentrate under test, and calculate the number of enzyme units per gram of added material. This represents the degree of proteolytic activity of the material.

Some typical values obtained by the use of this method are given in Table V.

TABLE V

PROTEOLYTIC ACTIVITY OF CERTAIN SUBSTANCES

Material	Proteolytic activity enzyme units per gram
Northwestern patent flour " A "	0.010
" " " " B "	0.0072
" " " " C "	0.0034
Low diastatic malt extract " A "	0.16
" " " " " B "	0.22
High " " "	2.7
Papain	110.0

Summary

Using an easy replicable dough of uniform consistency as a standard substrate, a method of determining proteolytic activity is described. The *development energy* (D.E.) of the dough is defined as that energy, expressed as the product of degrees Brabender by the time: —°B.-minutes, required to develop the standard test dough to its maximum consistency. The *rate* of *decrease* of D.E., is found to be proportional to the time and to the concentration of proteolyst, within certain limits. One proteolytic enzyme unit has been tentatively defined as that amount of enzyme which, when present in the standard dough will produce an initial rate of decrease of D.E. of 1000° B.-minutes per hour. The degree of proteolytic activity of a substance is taken as the number of enzyme units per gram of material. So defined the unit is believed to be independent of the flour used for the preparation of the standard dough.

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THE NATURE OF THE PIGMENTS OF THE GASOLINE EXTRACT OF WHEAT [1]

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The pigments extractable from wheat or flour by means of gasoline or other hydrocarbon solvents have been of considerable commercial importance since they to a large degree influence the color of the finished products such as bread or macaroni.

Wesener and Teller (1911) considered the major pigment of wheat flour to be similar to if not identical with carotene. Monier-Williams (1912) compared the absorption spectra of the gasoline extract of flour with that of carrot carotene and concluded the two were identical. Ferrari (1929) compared the absorption spectra of gasoline extracts of flour with that of carotene from carrots. The bands of the gasoline extract were slightly shifted towards the violet when compared with those of carrot carotene. He attempted an assay of the pigments by means of the Tswett absorption method, but could not find any xanthophyll. This coupled with the fact that xanthophyll is rather insoluble in hydrocarbon solvents led him to consider that carotene is the major pigment of the gasoline extract of wheat flour.

Coward (1924) and Hanna (1931) in studies of wheat leaves by two distinct methods found xanthophyll to be the major carotenoid pigment and carotene the minor pigment. Palmer (1922) found wheat bran to

contain xanthophyll. Bowden and Moore (1933) have isolated xantho-
phyll from the oil of the embryo of wheat.

Karrer and Ishikawa (1930) have shown that xanthophyll is very
commonly found as esters of the fatty acids. The xanthophyll esters
are more soluble in hydrocarbon solvents than in alcohols. Kuhn and
Brockmann (1932) have shown that while free xanthophyll was prefer-
entially dissolved in aqueous methanol out of an aqueous methanol-ben-
zine system, yet the xanthophyll esters, helenien and physalien, accom-
panied the carotene and lycopene into the benzine layer. However, if
the esters were saponified then the resulting free xanthophyll and fatty
acids would migrate to the aqueous methanol layer. They also found
α-carotene and leaf xanthophyll or lutein to have the same absorption
spectra, while β-carotene and zeaxanthin were likewise identical. Win-
terstein and Ehrenberg (1932) have shown that in mixtures of carote-
noid pigments the individual absorption bands of each pigment do not
appear, but that bands in intermediate positions are formed.

In view of these more recent findings it seemed advisable to attempt
the fractionation of the pigments of the gasoline extract of wheat. The
wheat extracts were found to be too dilute to give definite bands in the
Tswett CaCO₃ tower, although a part of the pigment was adsorbed in
passing through a 42″ tower.

The next step was the application of the Willstätter and Stoll (1913)
method of separating carotene from xanthophyll by means of the solubil-
ities of the pigments. When the crude naphtha extracts of wheat were
shaken with 90% methanol the separation was vague and indefinite.
After some preliminary work it was found that if the naphtha extract
was shaken with alkaline aqueous methanol the separation was sharp and
clear. It was found that two saponifications with one-half volume of
11% KOH in 85% methanol for 30 minutes each in a shaking machine
followed by two extractions with 90% methanol would remove all the
xanthophyll, and the naphtha layer would then only contain carotene and
any lycopene that might be present. It was found convenient to break
emulsions by means of the centrifuge and then to syphon off the super-
natent naphtha layer. This system of extraction is shown graphically
in Figure 1 in which the carotene fraction is listed as II.

The efficiency of this method of separation was checked by making
the separation upon solutions of pure carrot carotene secured from Dr.
Ferrari. Upon two separate trials conducted at different times the re-
covery of carotene as measured by the spectrophotometer was a little
more than 100%, the discrepancy was probably due to evaporation of the
naphtha and to solution of some of the naphtha in the methanol.

The carotene fractions of the naphtha extracts of four lots of wheat
were determined. The results are given in Table I. It will be noted

Separation of the Pigments of the Naphtha Extract of Wheat.

Freshly Ground Wheat
Extract 16 hrs. with
Naphtha
Centrifuge

Clear Naphtha Extract ⟶ Total Carotenoids I

Saponify with Alkaline MeOH in Cold
Centrifuge

Alkaline MeOH Layer
Naphtha Layer

Resaponified
Centrifuge

Alkaline MeOH Layer
Naphtha Layer

Combined MeOH Extracts   Wash Twice with 90% MeOH
Dilute with H₂O

Extract with Ether   MeOH Washings
Ether Layer ⟶ Evap. in Vacuo   Naphtha Layer ⟶ Centrifuge ⟶ Carotene II
MeOH Layer   III
Dissolved in Abs. Alcohol ⟶ Filter ⟶ Filtrate ⟶ Xanthophyll VII
Precipitate

Re-extract with Ether Twice   ⟶ Dissolve in CS₂ ⟶ Reddish-
Ether Layers ⟶ Evap. in Vacuo   Precipitate   Orange
MeOH Layer   IV-V   Pigment VIII
Dissolved in Abs. Alcohol ⟶ Filter

Stand for 1 Week   Filtrate ⟶ CS₂ ⟶ Xanthophyll VI
Extract with Ether   Alcohol

Ether Layer ⟶ Tricin IX
MeOH Layer

Re-extract with Ether
Ether Layer ⟶ Unidentified
MeOH Layer   Yellow Pigment X

⟶ Flavones XI

Fig. 1.

that the lightly pigmented Marquis wheat had only 12.8% of its pigments as carotene, while the heavily pigmented Minturki contained 34.8% of its pigments as carotene.

TABLE I

RELATIVE AMOUNTS OF CAROTENE IN NAPHTHA EXTRACTS OF CAROTENE AND OF WHOLE WHEAT AFTER REMOVAL OF XANTHOPHYLLS AS COMPARED TO ORIGINAL CAROTENOID CONCENTRATION

Material used	Carotene as per cent of total pigments
	%
Carotene solution from carrots (Ferrari)	107.6
Carotene solution from carrots (Ferrari)	103.5
Naphtha extract Marquis Wheat (1931)	12.8
Naphtha extract Minturki Wheat (1931)	34.8
Naphtha extract Mindum Wheat (1931)	21.5
Naphtha extract Mindum Wheat (1933)	16.7

The transmittancy of light at various wave lengths in the visible spectrum of the carotene fraction of ground Mindum durum wheat of the 1933 crop was determined. The results are given in Table II and more graphically in Figure 2. The locations of two absorption bands

○—○ Carotene fraction in naphtha.

●—● Xanthophyll fraction in alcohol.
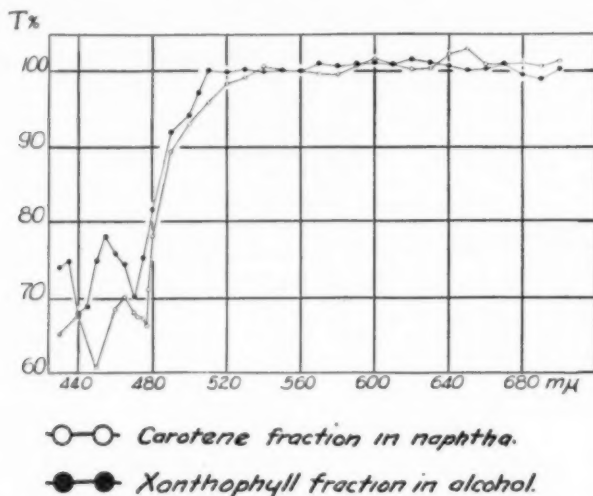
Fig. 2. Transmittancy-wave length graph for the carotene fraction (II) and the xanthophyll fraction (VII) of Mindum durum wheat.

are defined with evidence of a third just below the visible range. The instrument used was a Keuffel and Esser Color Analyser using a 10 cm. cell. From a study of the location of the bands it does not seem likely that any appreciable quantity of lycopene was present, and that the carotene may have been a mixture of the α and β forms.

TABLE II

TRANSMITTANCY VALUES FOR THE CAROTENE FRACTION OF GROUND WHOLE MINDUM DURUM WHEAT

Wave length, $m\mu$	Transmittancy, 10 cm. cell	Wave length, $m\mu$	Transmittancy, 10 cm. cell	Wave length, $m\mu$	Transmittancy, 10 cm. cell
430	65.1	490	89.5	600	101.5
440	67.9	500	93.0	610	100.8
450	60.7	510	95.8	620	100.2
460	68.6	520	98.3	630	100.3
465	70.1	530	99.1	640	102.3
470	68.1	540	100.7	650	103.0
472	67.6	550	100.0	660	100.8
475	67.3	560	99.8	670	101.2
477	66.3	570	99.6	680	101.2
478	71.2	580	99.5	690	100.7
480	78.2	590	100.7	700	101.3

The various methyl alcohol fractions from the separation of the carotene were combined as shown in Figure 1. These were diluted with about half volume of water and extracted with successive 50 cc. portions of ether until colorless. Three extractions were enough. The ether fractions were then vacuum concentrated at temperatures below 60° C. to a syrup. Absolute ethyl alcohol was then added to the syrup. A clear yellow solution resulted with quantities of a chocolate brown precipitate which was insoluble in the alcohol. The clear yellow solutions from the above extraction were shaken with CS_2 and small quantities of a reddish brown pigment (VIII) removed. The absolute alcohol now appeared to contain only relatively pure xanthophyll (VI and VII) as pigments.

The absorption spectra of this xanthophyll (VII) was determined as for the carotene. The results are given in Table III and Figure 2. An inspection of Figure 2 indicates that the bands are shifted towards the violet in the xanthophyll as compared with the carotene fraction from the same wheat.

TABLE III

TRANSMITTANCY VALUES FOR XANTHOPHYLL FRACTION OF GROUND WHOLE MINDUM DURUM WHEAT (DILUTED 1–4 WITH ABSOLUTE ALCOHOL)

Wave length, $m\mu$	Transmittancy, 10 cm. cell	Wave length, $m\mu$	Transmittancy, 10 cm. cell	Wave length, $m\mu$	Transmittancy, 10 cm. cell
430	74.1	490	92.0	600	101.2
435	74.9	500	94.2	610	100.8
440	68.1	505	97.2	620	101.5
445	68.9	510	100.1	630	101.0
450	75.0	520	99.9	640	100.7
455	78.1	530	100.3	650	100.2
460	75.9	540	99.9	660	100.3
465	74.5	550	100.1	670	101.0
470	70.2	560	100.0	680	99.5
475	75.3	570	101.0	690	99.0
480	81.6	580	100.7	700	100.3
		590	101.0		

The chocolate brown precipitate from the separation of xanthophyll was very highly insoluble. It appeared to be similar to a sterol with a brownish pigment adsorbed upon it. A portion of this pigment could be dissolved in CS_2 forming an orange-red solution (VIII). This solution had no indicator properties, but was positive to the phenol reagent of Folin and Denis. It gave a brown coloration and voluminous precipitates with ferric iron and lead salts. The transmittancy values for different wave lengths throughout the visible spectrum are given in Table IV and graphically in Figure 3. This indicated a maximum of absorption at 460 $m\mu$ with rather continuously decreasing absorption of
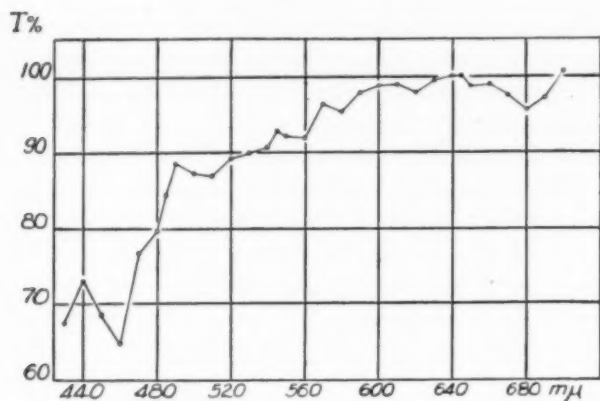
Fig. 3. Transmittancy-wave length graph for the orange-red fraction (VIII), in CS₂, of the naphtha extract of Mindum durum wheat. Data from Table III.

light to 600 mμ with a minor absorption in the red at 680 mμ. The exact nature of this pigment is as yet unknown.

TABLE IV

TRANSMITTANCY VALUES FOR EXTRACT VIII OF GROUND WHOLE MINDUM DURUM WHEAT

Wave length, mμ	Transmittancy, 10 cm. cell	Wave length, mμ	Transmittancy, 10 cm. cell	Wave length, mμ	Transmittancy, 10 cm. cell
430	67.5	520	89.3	610	99.1
440	73.0	530	90.0	620	98.1
450	68.6	540	90.7	630	99.6
460	64.8	545	92.8	640	100.1
470	76.6	550	92.1	645	100.1
480	79.7	560	91.9	650	98.8
485	84.4	570	96.5	660	99.2
490	88.7	580	95.5	670	97.6
500	87.3	590	98.1	680	95.7
510	86.9	600	98.9	690	97.3
				700	100.7

The colorless aqueous methanol extracts, from which the xantho-phyll and the brownish pigment were extracted, were allowed to stand in the dark for a week. At the end of this time the solutions were found to be brownish yellow in color. An extraction with ether yielded a brownish yellow solution which exhibited the characteristic reactions of the Tricin (IX) of Anderson (1931). A second extraction with ether gave a lemon yellow solution (X) which was negative to the phenol reagent. The chemical nature of this pigment is as yet unknown. The residual methanol solution was still brownish yellow and probably contained flavones (XI) less ether soluble than tricin. The intensity

of coloration of this residual solution continued to increase during six months storage.

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THE PIGMENTS OF THE DILUTE ALCOHOL OR ACETONE EXTRACT OF WHOLE WHEAT MEAL [1]

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While the carotenoid pigments of wheat have received the major attention of the cereal chemist, the existence of appreciable quantities of pigments of other types must not be overlooked. One of the major groups of pigments of plants is the flavone group. The members of this group are phenolic in character and can be classed as water soluble in contrast to the fat solubility of the carotenoids.

In 1925, Collison reported a qualitative test for vanillan in wheat leaves. Kent-Jones and Herd (1927) presented a method for extracting pigments from wheat flour by means of extraction with dilute (67%) alkaline methanol. They obtained a brownish-yellow extract which had strong indicator properties, being brownish-yellow in alkaline solution and nearly colorless in acid solutions. They found the quantity of this pigment to decrease with increasing refinement of the flour, and assumed it to be in bran flecks. They decided that this pigment was xanthophyll, because of its solubility in dilute methanol, and also because Palmer (1922) had found xanthophyll in wheat bran. But the accuracy of the identification of this pigment as xanthophyll is open to very serious questioning. Palmer describes xanthophyll in dilute solutions as being greenish, and Kent-Jones and Herd's pigment is brownish. All other work reported in the literature indicates that xanthophylls are unaffected by alkalies, and only exhibit a color change in the presence of strong acids. The extracts of Kent-Jones and Herd may have contained some xanthophyll, but the pigment they described was certainly not xanthophyll.

Simpson (1928) in a communication to Dr. C. H. Bailey reported the presence of at least two flavone pigments in the dilute methanol extract of bran. This is the first recognition of the presence of flavone pigments in wheat, although Shibata and Nagai (1916) had shown that the flavone compounds are universally found among plants. Their

studies took in 242 species of plants, including 64 families and 190 genera, and in no case did they fail to find flavone pigments to be present.

The author in 1929, during a study of the acidity of flours of varying refinements by the Balland procedure, noted that the depth of yellow color in the 85% alcohol extracts of flour was apparently a function of the grade of flour. The extracts of the highest grade flours had very little color, while the extracts of flour of lower grades were deeply pigmented. It was possible to accurately score flours for grade by a visual comparison of the depth of color in the alcoholic extract with the use of a colorimeter or other instrument.

Newton and Anderson (1929) have shown that ether-soluble phenolic compounds which appear to be flavone pigments are found in the leaves of several varieties and species of wheat. As the branny layers of wheat are mother plant tissue and neither embryo nor reserve food material, it seems logical to expect to find these ether-soluble flavone pigments in the bran, and they should appear in the gasoline extract, but should migrate to an aqueous alkaline alcohol layer.

Anderson and Perkin (1931) isolated one of the ether-soluble flavone pigments from wheat. This pigment they named "tricin." Anderson (1932) presented the following formula for tricin:
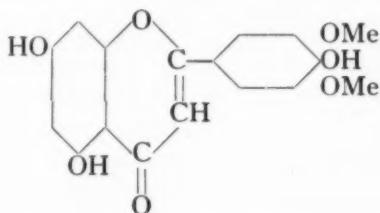
He noted other flavones less soluble in ether than tricin to be present in wheat.

Schulerud (1933) presented a modification of Kent-Jones and Herd's method of estimating the quantity of dilute alcohol-soluble pigments in mill products and the bread from these products. He found the pigments to be present in largest quantity in the bran and in least quantity in the most refined mill streams. In a second paper (1933a) he gives data on the spectral absorption of light by his extracts. He finds the maximum coefficient of extinction at 430 mμ and minimum extinction at 610 mμ and above. The line of extinction is fairly smooth. It is similar to, but a little farther toward the red than Ferrari's (1929) bran extract. This may be due to the alkalinity of Schulerud's extracts. Schulerud shows that the extinction coefficient is constant at a minimum value up to pH 7.2 and then increases in a linear manner to pH 9.1

when it again becomes linear. Shibata and Kimotsuki (1923) have shown that many flavones have a major absorption band at 450 mμ.

Lewicki (1929) found anthocyanin in the cell contents of the outer epidermis of wheat. He found flavones to exist both in free state and as gucosides. He has also found two types of tannins.

Experimental

This phase of the work was carried out before Anderson (1931, 1932) had published his methods for the isolation and identification of the flavone pigment tricin. In order to study the reason for the bran extracts of Ferrari's (1929) differing from the absorption spectra of flour extracts and of carotene solutions, a study was made of the dilute-alcohol-soluble pigments of whole wheat. It was found that 50% to 67% alcohol was an excellent solvent for pigments of the flavone type. These pigments in the slightly acid extract are yellow with a slight brownish tint which is quite distinct from the orange-yellow of carotene or the lemon-yellow of xanthophyll. When the alcoholic extract is made alkaline the solution becomes an intense greenish-yellow. Upon standing a few days the alkaline solution becomes dark brownish-yellow. The dilute alcohol apparently extracts very little of the carotenoid pigments as only a small portion migrates to the naphtha layer when naphtha is mixed with the dilute alcohol.

Fifty percent aqueous acetone was found to be quite similar to dilute alcohol as a solvent for flavone pigments, and the extracts appeared to be somewhat clearer and more sparkling. An extract made by extracting 20 g. of ground Mindum durum wheat with 100 cc. 50% acetone was centrifuged and siphoned into a 1 cm. cell, and transmittancy was determined over the visible scale of the Keuffel and Esser color analyser. For greater accuracy the transmittancy at 438.5 mμ was determined by means of a 10 cm. cell and the light of the mercury arc. The transmittancy values were calculated to extinction coefficients for each wave length, for this particular extract. The values are given in Table I and shown graphically in Figure 1. Upon examination of the curve it will be noted that there is heavy absorption at 435.8 mμ which drops to a minimum value at 480 mμ with a minor band at 525 mμ and possibly one at 565 mμ. The absorption of light ceases at about 590 mμ. This curve is remarkably similar to that obtained by Ferrari (1929) on a similar examination of the gasoline extracts of wheat bran.

These results made it appear desirable to investigate the absorption spectra of these extracts in the ultra-violet. As the Division of Biochemistry was not equipped for this work, arrangements were made with the Department of Physics, of the University of Minnesota, to make the determinations with their equipment. This portion of the

TABLE I

EXTINCTION COEFFICIENT VALUES OF AQUEOUS ACETONE EXTRACT OF GROUND
WHOLE MINDUM DURUM WHEAT

One Centimeter Cell (Except at 435.8 mμ 10 centimeter used)

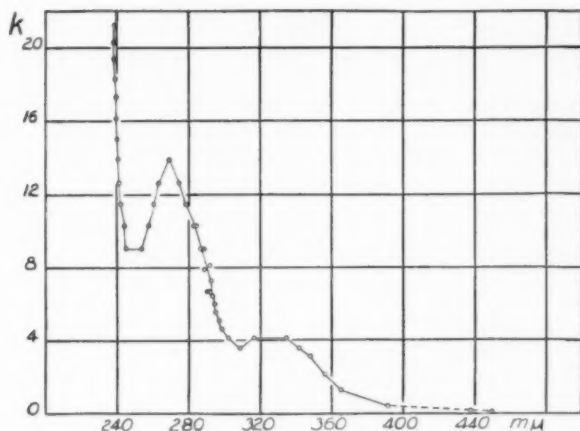| Wave length mμ	Extinction coefficient	Wave length mμ	Extinction coefficient	Wave length mμ	Extinction coefficient
435.8	0.19173	530	0.06521	620	0.00903
450	.10278	540	.06333	630	.01326
460	.07882	550	.02284	640	.00475
470	.03730	560	.04336	650	.01620
480	.01242	570	.04139	660	.01326
490	.05077	580	.03181	670	.01326
500	.05077	590	.00647	680	.00903
510	.05538	600	.01157	690	.01326
520	.06521	610	.01157	700	.00475
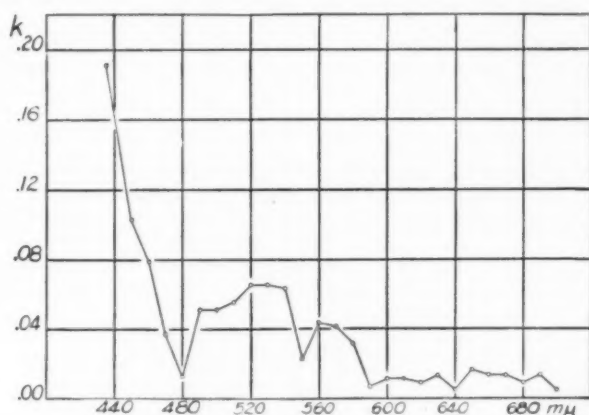
Fig. 1. Extinction coefficient-wave length graph for the aqueous-acetone extract of Mindum durum wheat. Data from Table I.

work was carried out by Mr. W. Wetzel of that department. The equipment used in this work was a quartz Hilger type E–3 spectrograph, a Judd Lewis sector-photometer, and an under-water spark between tungsten electrodes. The photometer divides the light into two equal beams one of which passes through the sector disc. The intensity of the beam passing the sector disc is under the control of the operator and can be set to any desired percent of the other beam. The extract was placed in a 2 cm. quartz cell in the constant beam, and solvent was placed in a similar cell in the variable beam. Thus by taking the spectral photographs of the light passing through the extract and of the reference beam at different settings of the sector a series of photographs was obtained on which, at any one photometer setting, points of equal intensity between the photograph of the light passing through the extract

and the reference beam can be determined.   The photometer is calibrated in terms of the extinction coefficient, which is the logarithm to base 10 of the reciprocal of transmittancy.

After some preliminary work it was found that acetone was unsuitable as a solvent for transmittancy studies in the ultra-violet as it has a very pronounced absorption spectrum of its own.   So an extract of the same wheat was prepared using 67% ethyl alcohol as a solvent.   This extract was adjusted so as to have essentially the same transmittancy at 435.8 mμ as the acetone extract.   This extract was well suited for the purpose.   It was found that the absorption increased so rapidly with decreasing wave length that the original solution was too concentrated, so it was diluted one to nine, and for work below 300 mμ was diluted 1 to 24.   The data for points of equal intensity are given in Table II. The extinction coefficient wave length curve is shown in Figure 2.   The extinction coefficient is on a scale only one-hundredth as great as in Figure 1.   When allowance is made for this difference it will be seen that there is very good agreement between the curves, and that one can

TABLE II

EXTINCTION COEFFICIENT WAVE LENGTH DATA FOR THE AQUEOUS ALCOHOL EXTRACT OF MINDUM DURUM WHEAT

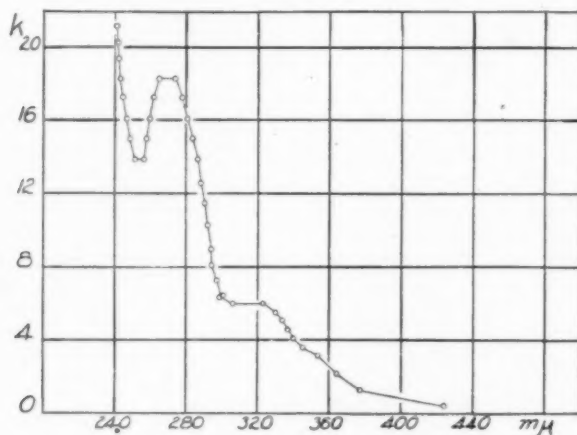| Extinction coefficient	Wave length in mμ at equal blackening			
	Dilution 1–9			
.40	379.0			
1.25	365.5			
2.15	356.0			
3.15	348.0			
3.60	341.5	308.5		
4.10	334.5	316.5	302.0	
4.60			298.5	
5.05			297.0	
5.55			295.7	
6.00			294.5	
6.45			293.7	
7.30			292.5	
8.10			292.0	
	Dilution 1–24			
6.63	292.0			
9.00	288.5			
10.3	283.0	257.5	243.5	
11.5	278.5	260.0	242.0	
12.6	274.5	263.0	241.0	
13.9	269.0		240.5	
15.0			240.0	
16.1			239.5	
17.3			239.2	
18.3			239.0	
19.4			238.5	
20.3			238.4	
21.2			238.3	

Fig. 2.   Extinction coefficient-wave length graph for the aqueous-alcoholic extract of Mindum durum wheat.   Data from Table II, except the 2 lower points from Table I.

be considered as essentially a continuation of the other. At a wave length of 237 mμ the absorption is rising almost vertically. From this point to 590μ the general shape is that of an exponential curve. However at 269 mμ there is a very strong absorption band, and there is another at 325 mμ. It is not clear from an inspection of these curves whether only one substance is responsible, or whether the exponential type of smooth curve is representative of one substance and the superimposed bands at 269, 325, and 565 mμ are caused by the presence of one or more additional substances.

The absorption spectrum of the same solution, when made alkaline with concentrated sodium hydroxide was then determined. The data are given in Table III and the extinction coefficient-wave length curve is given in Figure 3. When this curve is compared with the one in Figure 7 it will be noted that the addition of alkali has not shifted the position of the absorption bands at 269 and at 325 mμ. The increase in the intensity of these bands seems entirely due to the shifting of the basic exponential curve towards the red end of the spectrum. With an extinction coefficient of 21 the shift is only 3 mμ, but by the time the extinction coefficient has been lowered to 0.4 the shift is from 379 mμ to 424 mμ. This accounts for the increase in apparent pigment concentration when such solutions are made alkaline. It had been noted that when the transmittancy of the solution had been measured at 435.8 mμ the apparent pigment concentration calculated as carotene (which is only an approximation) was quadrupled when the solution was made alkaline.

Kent-Jones and Herd (1927) considered that their method of extracting these pigments gave them a measure of the amount of red bran pigments contaminating the sample of flour under examination. In

TABLE III

EXTINCTION COEFFICIENT WAVE LENGTH DATA FOR THE ALKALINE AQUEOUS ALCOHOL EXTRACT OF MINDUM DURUM WHEAT

Extinction coefficient	Wave length in mμ at equal blackening		
	Dilution 1–9		
.40	424.0		
1.25	377.5		
2.15	364.0		
3.15	353.5		
3.60	345.5		
4.10	340.0		
4.60	337.0		
5.05	334.0		
5.55	330.0		
6.00	323.0	306.0	
6.45		301.0	
7.30		297.0	
8.10		294.5	
	Dilution 1–24		
6.63	299.0		
9.00	294.0		
10.3	292.0		
11.5	290.0		
12.6	288.5		
13.9	286.5	256.0	251.2
15.0	283.5	257.8	248.5
16.1	280.3	259.5	246.5
17.3	277.8	261.5	244.5
18.3	274.0	265.0	242.8
19.4			242.2
20.3			241.5
21.2			241.1

Fig. 3. Extinction coefficient-wave length graph for the alkaline aqueous-alcoholic extract of Mindum durum wheat. Data from Table III.

order to test this hypothesis, a sample of red bran was treated with pancreatin until all adhering starch was removed. It was carefully washed, and then subjected to the extraction procedure of Kent-Jones and Herd. Their method was followed exactly. The bran yielded a large quantity of pigments, but its appearance was not changed when compared with an unextracted portion of the same bran. Their technic was applied to samples of ground Marquis, Mindum, and Dicklow wheat. All of these wheats regardless of bran color yielded approximately equal quantities of the flavone pigments (xanthophyll of Kent-Jones and Herd).

While working with the procedure of Kent-Jones and Herd, it was noted that an alkaline alcoholic or acetone extract was much more efficient than a neutral solvent in extracting flavone pigments from wheat. Repeated extractions of the same sample appeared to continually extract more pigment. This suggests that the flavone pigments are tied up in chemical linkages with other substances. These linkages are apparently of such a nature as to be readily broken with alkalies. The flavones have long been known to exist as glucosides and from the preceding study of the gasoline-extractable pigments of wheat it appears at least possible that they will be found as esters of the fatty acids.

A sample of ground Marquis wheat was extracted successively with gasoline and neutral acetone until it was free of pigments readily soluble in those solvents. It was twice extracted with 0.4% NaOH in 67% aqueous acetone solution overnight, and the extracts discarded. The residue was set aside in contact with another portion of alkaline acetone in the dark for two years at room temperatures. At the end of this time the extract was found to be dark red, almost black by reflected light. (A similar extract of Mindum durum was still yellow.) This extract was decanted and ether added until there was a distinct layering. The water layer contained a very dark red color, which was insoluble in carbon bisulphide. After evaporation to a small volume on a steam bath it was filtered, yielding a clear very dark red solution. This is very similar to the red pigment obtained by Simpson (1928) from the alkaline filtrates of crude fiber determinations, which he considers to be the red pigment of the bran. However, it must be remembered that a prolonged alkaline acetone extraction gives very good opportunity for condensation or polymerisation reactions to take place so the pigments from this study may not be normal constituents of wheat. This dark red extract reacts positively with the Folin and Denis reagent, indicating at least that the extract contains phenols. The red pigment has no pronounced indicator properties and is not precipitated by acids. The ether extract of the original acetone solution showed the presence of phenolic

pigments in relatively large quantities of the same general types as those previously described, namely a yellow flavone extract soluble in naphtha, a deeper yellow naphtha insoluble flavone pigment, and the carbon disulphide-soluble reddish-orange pigment of phenolic character, but having no pronounced indicator properties. Evidences of non-phenolic yellow pigments were obtained in this extract.

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DETERMINATION OF THE CAROTENOID PIGMENT CONCENTRATION OF SMALL SAMPLES OF WHOLE WHEAT [1]

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Since the carotenoid pigment content of commercial wheats is a factor in determining their market value and utility, it is of importance to the plant breeder in the selection of new varieties to give this character much consideration. If lines having undesirable pigment concentration could be eliminated early in the breeding program much wasted time and expense could be avoided. So the development of a method of accurately determining the carotenoid pigment content of a small sample of whole wheat becomes the task of the experiment station chemist.

Ferrari (1929 and 1933) has developed a highly accurate method for the determination of the total carotenoid pigment of wheat flour. Whiteside (1931) has shown that the carotenoid content of finely ground whole wheat meal is essentially the same as that of patent flour experimentally milled from the same wheat. Also Whiteside has shown that the carotenoid content of patent flour is correlated with the crumb color score of the bread baked from the same flour. Markley and Bailey (1933) have shown that the crumb color score for flour experimentally milled is correlated with the same score for flour milled from the same wheat but in a commercial mill.

Determination of Carotenoid Pigments

The determination of the total carotenoid pigments of whole wheat was made substantially in the same manner as reported by Ferrari (1933) for flour after grinding the sample in accordance with the recommendations of Whiteside (1931). If at least six grams of material was available in all samples in a given series, the determination was conducted with the exact technic recommended by Ferrari. These samples were ground on a steel burr mill (Seck) with very finely corrugated burrs. An average distribution of particles as to size after a single grinding was:

80% passing a 50 gritz gauze,
40% passing a 10 XX flour silk.

A 5 g. sample of the meal was weighed into a dry bottle with a tight fitting ground glass stopper. Weighing was done on a triple beam balance with a sensitivity of 5 mg. Then 50 cc. of a mixture of 93% cleaner's naphtha (as specified by Ferrari) and 7% absolute alcohol was pipetted into the bottle, and the bottle placed in a shaking machine located in a dark chamber and shaken 20 minutes. The bottle was then allowed to stand overnight and was reshaken for 20 minutes in the morning. The suspension was then poured into a centrifuge tube. Tapered bottom tubes without lips are preferable. The samples were then centrifuged for 10 to 20 minutes at a rate of speed comparable to 3000 r.p.m. on a ten inch diameter centrifuge head. It was better to allow plenty of flour or meal to accompany the naphtha into the centrifuge, as at this speed the flour was packed into a solid cake at the bottom, and in tapered tubes was relatively resistant to disturbances, whereas if only a little meal or flour was in the tube it could not form a cake and was easily disturbed. The clear naphtha solution was siphoned by means of a capillary siphon into clean dry bottles. The extract was poured from these bottles into the cell of the spectrophotometer.

If only from one-half to one gram of wheat was available the technic was slightly varied. By taking precautions it was possible to take a sample of wheat weighing 0.55 g., grind it very fine on the Seck burr mill, and recover 0.50 g. The loss rarely exceeded 0.1 g. A sample of 0.50, 0.75, or 1.00 g. of meal was weighed on the triple beam balance, and transferred to a clean, dry bottle with tight fitting cover. The size of bottle was governed by bottom area. Bottles 1.75 inches in diameter were used for 0.50 g. samples, 2 inches for 0.75 g. samples, and 2.25 inch bottles for the one-gram samples. In this way depth of solution was kept very nearly constant. Ten cubic centimeters of the naphtha-alcohol mixture mentioned above was used for a 0.50 g. sample, 15 cc. for a 0.75 g. sample, and 20 cc. for a 1.00 g. sample. The naphtha-alcohol mixture was pipetted by ordinary pipettes. This mixture did not give off offensive fumes if only small lots were handled. The steam from the pipette was directed around the neck and sides first to wash down particles, and then it was directed through the sample in the bottle in such a way as to break up aggregates. The bottle was never shaken at the start of an extraction, because the size of sample was so small, and the ratio of solvent to sample so large, that the amount that would remain on the side of the bottle after shaking would have introduced serious errors. The bottle was allowed to stand overnight.

In the morning the bottle was well shaken, and the contents poured into a 15 cc. tapered centrifuge tube, closed with a cork, and centrifuged as directed above.   The clear extract was siphoned directly into a 5 cm. double cell of the type described and illustrated by Ferrari (1933).

Variability in the Determination of the Carotenoid Pigments of Whole Wheat

In a problem involving optical measurements of extracts prepared from relatively small samples of biological material the amount of variability is of great importance.   Accurate conclusions cannot be safely drawn without a knowledge of both systematic and random variability in the system.   This variability can be grouped into three categories: First, variability due to the limitations of the optical instrument in use and to the eye of the operator; second, variability due to sampling and the operations involved in preparing the extract for measurement; and third, variability in the biological material (this variability may be due to either or both genetic and environmental causes).

The variability due to the limitations of the instrument, namely the Bausch and Lomb photometer previously described, had not been determined, although Ferrari (1929) had made a careful study of the variability in determinations made under similar conditions with a Keuffel and Esser color analyser.

In the evaluation of that portion of the variability of determinations of carotenoid concentration of wheat due to variability of the instrument and of the eye of the operator, it was necessary to replicate readings

TABLE I

VARIABILITY IN DETERMINATIONS OF THE TRANSMITTANCY OF SOLUTIONS AT A WAVE LENGTH OF 438.5 $m\mu$ WITH THE BAUSCH AND LOMB PHOTOMETER

| | (50 replicated readings represented in each sample) Percent transmittancy | | Standard error | |
| | | | | |
Mean	Standard deviation	Coefficient of variation	$n = 10$	$n = 16$
24.72	0.6940	2.8073	0.2195	0.1735
25.98	0.7871	3.0298	0.2489	0.1968
39.24	1.1412	2.9083	0.3609	0.2853
45.66	1.4228	3.1161	0.4500	0.3557
61.68	1.7600	2.8534	0.5566	0.4400
67.88	1.5315	2.2562	0.4844	0.3829
68.04	1.3994	2.0568	0.4426	0.3499
73.80	1.4142	1.9163	0.4473	0.3536
75.78	2.0004	2.6397	0.6326	0.5001
76.54	2.3427	3.0608	0.7409	0.5857
77.24	2.0934	2.7103	0.6621	0.5234
83.08	1.9681	2.3690	0.6224	0.4920

many more times than in the course of a normal reading of the sample. Therefore in this work the study of the variability of the Bausch and Lomb photometer was made by making 50 replicated readings upon 12 extracts having transmittancy values scattered at random over the range normally used in the course of these investigations. Table I gives the means, standard deviations, and coefficients of variation of these twelve sets of readings. The standard errors of the means were calculated for an " n " of 10 and an " n " of 16. In Figure 1 standard errors for the

●   *Variability as the standard deviation.*

◐   *Variability as coefficient of variation.*

○   *Variability as standard error (n=10).*

◑   *Variability as standard error (n=16).*
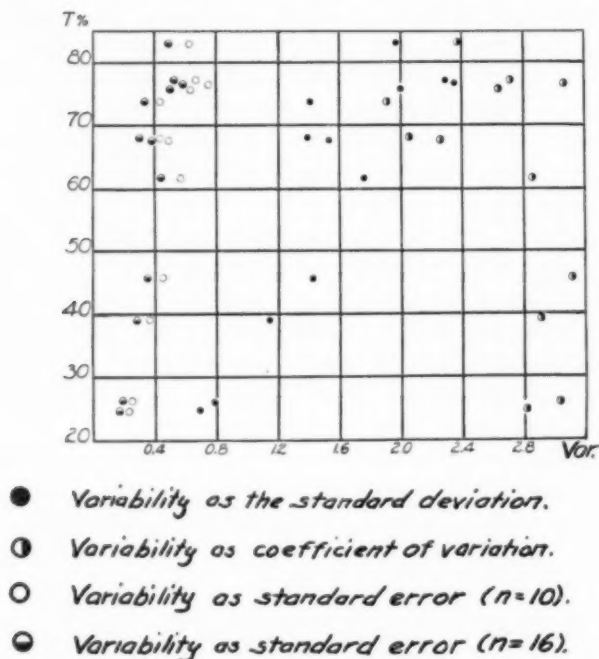
Fig. 1. Variability in reading the Bausch and Lomb photometer at different levels of transmittancy. 50 replicated readings at each level.

means of 1, 10, and 16 readings are plotted against mean transmittancy. The graph shows that as the concentration of pigments decreased the error increased. This was in fairly good agreement with the findings of Ferrari. In the lower ranges of transmittancy the errors for the Bausch and Lomb photometer were almost identical with the values found by Ferrari for the Keuffel and Esser instrument, but in the higher ranges of transmittancy the Bausch and Lomb instrument was subject to greater errors. This was probably due to the construction of the instrument as it was very difficult to set the Bausch and Lomb instrument so as to have uniform illumination throughout the entire field of view. The coefficient of correlation between mean-percent-transmit-

tancy and the standard errors of the mean-percent-transmittancy was $r = + .8819 \pm .0641$. The deviation of this coefficient from unity indicated that the operator's eye varied from time to time in sensitivity. In view of the greater variability of this instrument eight readings were taken with the cell in each position, thus giving sixteen readings for each sample.

In order to measure the variability of the entire analytical procedure, a very uniform sample of Thatcher wheat of the 1933 crop was carefully divided into 36 two-gram portions. On one day 18 of these portions were ground in the usual manner and one-gram samples extracted, and the transmittancy of the naphtha-alcohol extracts determined. The mean pigment concentration as carotene in parts per million was 3.3056, with a standard deviation of 0.2272, and a coefficient of variation of 6.8743. The following day the second series of 18 samples was ground and extracted. The mean on this series was 3.4167, with a standard deviation of 0.2892, and a coefficient of variation of 8.4631.

A sample of damp, mixed, feed Durum wheat was very carefully divided into 18 portions with a Boerner sampler. These samples were individually ground. The grinding was attended with difficulties owing to the damp wheat clogging the burrs. The coefficient of variation in this series was 26.835. This indicated that the small sample method was not suited to the analysis of material having a large variation in pigment concentration. To properly analyse such material a fairly large sample should have been ground, a two-gram sample was too small. Some of the variability may have been due to uneven moisture losses during grinding. Damp wheat should be air dried at room temperatures before attempting to grind it on a mill of the Seck type.

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THE BUNZELL–BECKER MOISTURE DETERMINATION APPARATUS

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Introduction

Davis and Wise [1] in reporting on a five year summary of the Pioneer Section of the A. A. C. C. stated that for 99% of all the quantitative reports received the average range of error for moisture determination is 0.99%. In our check sample work for the New York Section of the A. A. C. C. we also found undesirably large variations for moisture in flour. The great differences as Davis and Wise state, are not due to change of moisture content of the small samples or heterogeneity of the larger samples. It seems obvious to us that the errors are due to the differences in methods used, particularly the individuality of different operators. The greatest error is probably introduced through absorption of moisture during the period of cooling, *i.e.*, the time elapsing between removal from drying oven and weighing.

The Bunzell-Becker apparatus was devised to do away, to a large extent, with the influence of variable operation of individual workers. The procedure is such that the results approach an absolute determination of water present in a sample regardless of location and individuality of operator.

Description of Apparatus

A is a specially designed glass chamber composed of the lower halfs of two large pyrex desiccators. Inside diameter at ground joint is 25 cm., thickness of wall is 10 mm. It is provided with a ground hole on top allowing for a tight joint with rubber stopper E. Dotted line on the right indicates a section of the wall polished on both sides to enable operator to make accurate observations. The desiccator is mounted on board B and the balance can be leveled with leveling screws C and C' (see leveling plummet Q). Heating is accomplished by means of three heating elements L, L' (not visible but placed immediately behind stand St), and M. These three units are arranged in series and were found to give the most desirable heat distribution. Current is supplied through cable J and transmitted through the wall by means of binding posts K and K' which are made vacuum tight by means of rubber washers. The

[1] Davis, C. F., and Wise, M. A five-year summary of the monthly check sample reports of the Pioneer Section of the A. A. C. C. Cereal Chem. 10: 203–212 (1933).

desiccator is evacuated through stopcock D and tube F. The small manometer G is attached to the end of the stopcock by means of the short rubber tubing H. Evacuation of desicator is made possible through small vent hole I. The automatic balance is mounted perma-
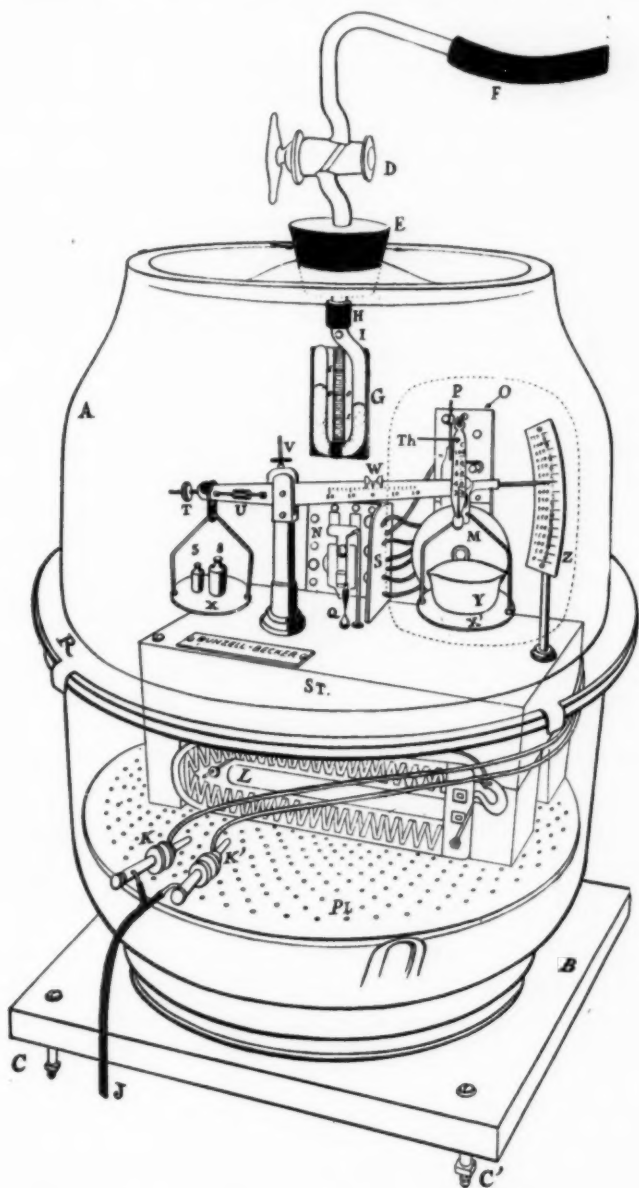
Diagram of Bunzell-Becker apparatus for moisture determination.

nently on stand *St*, which in turn is supported by perforated porcelain plate *Pl*. Platinum dishes such as *Y*, mounted on pan *X'*, are used for the moisture determination. These platinum dishes range in weight from 8.01 to 8.02 g. They can be counter-balanced by means of weight *8* (shown on pan *X*) and the rider *W*. Five grams of material weighed into dish are balanced by weight *5*, also shown on pan *X*. Adjustment screws *T*, *U*, and *V* enable operator to regulate the zero point and sensitiveness of the balance. The temperature regulation is made automatic through the relay *N* and thermoregulator *O*. Temperature setting is accomplished by means of the adjustable contact *P*. *Th* is a small thermometer readable to one degree. *S* is a mica plate protecting the relay against damage by the heating plate *M*. *R* is a metal ring provided with three prongs preventing slippage of upper part of chamber when not in use. Not shown in illustration is a polished glass plate of non-shatterable glass. This is placed immediately in front of apparatus and assures safety of operation.

Procedure

Five grams of flour, feed, cocoa, etc., is weighed into platinum dish and balance brought to the zero reading at bottom of scale *Z* by using weights *5* and *8* as well as rider *W*. Ground joint is coated with hard wax and top put in place. Stopcock *D* is open. Vacuum pump is turned on and cable *J* plugged into 110 AC outlet. Visible moisture loss begins immediately and readings of weight are made at intervals of five minutes. Completion of the determination requires from 30 to 45 minutes during which time the pressure falls to 5 mm. and the temperature rises to 90° C.

ILLUSTRATION OF TESTS BY BUNZELL-BECKER METHOD USING
FLOUR SAMPLE No. 7988

Time	Temperature	Reading	Moisture
Minutes	° *C.*		%
2:25	30	0	0.00
2:30	45	30	0.60
2:35	52	180	3.60
2:40	60	315	6.30
2:45	65	420	8.40
2:50	75	500	10.00
2:55	80	550	11.00
3:00	85	585	11.70
3:05	90	605	12.10
3:10	90	615	12.30
3:15	90	615	12.30
	Standard Drying Oven Method		
	(1 hour at 130° C.)		
12.46	12.34	12.18	Average 12.33

VARIABILITY IN EXPERIMENTAL BAKING USING HAND AND MACHINE MANIPULATION [1]

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(Read at the Annual Meeting, June, 1934)

Introduction

The difficulty of obtaining results that are reproducible within reasonable limits has been one of the main objections to the baking test. The wide variation in the volume of loaves baked from a single flour has necessitated extensive replication where accurate results were required. Complete mechanization of the baking procedure has often been suggested as a means of reducing this variability. However, satisfactory machines for punching and moulding doughs made from 100 g. of flour have not hitherto been available. About two years ago, at the University of Alberta, the construction of machines designed to handle this amount of dough was started, and the work has been continued at the National Research Laboratories at Ottawa.

Punching and Moulding Machines

The design of the punching machine offered no particular difficulties. The moulding machine, however, has been modified considerably since the construction of the first model. The principal difficulty was the sticking of the dough to the rolls when neither dusting flour nor grease was used. However this has now been overcome and both the punching and moulding machines operate perfectly in so far as actual handling of the dough is concerned. Since the last modification about 2,000 doughs have been put through the machines without any trouble. The machines in their present form are shown in Figure 1.

The punching machine has two sheet metal forms, tapering to a minimum diameter and expanding again, between which runs a canvas belt. The dough is carried through the tapering section *A*, being squeezed as the cross-sectional area becomes smaller, and is rounded up in the expanding section *B*.

In the moulding machine, the dough passes through a pair of sheeting rolls *C*, and is carried under the first curling roll *D*. The end of

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the dough sheet is turned up by the second curling roll E and then down again by the first curling roll D.   When the curling is completed the dough passes under the second curling roll E and moulding is completed in the compression chamber F.
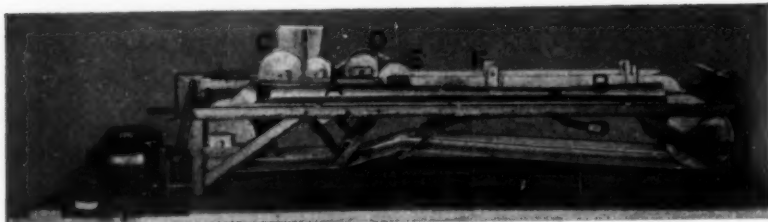

Fig. 1.   Punching and moulding machines.

The machines are fitted with devices to control the tension of the main belts but under the high humidity maintained in the baking laboratory these proved inadequate to compensate for the actual stretch and shrinkage of the canvas.   The machines are now being modified to obviate this difficulty.

Experimental

Plan of Experiment

In an experiment designed to study the variability of test baking using hand and machine manipulation, tests were made with five flours. Each flour was baked once a week for five weeks, each time on a different day of the week.   Thirty-six loaves were baked by the bromate formula each day, hand and machine manipulation being used alternately.   The volumes of the first four and the last four loaves were considered separately from the main experiment because of the chance that conditions, particularly in the oven, might differ at the beginning and end of the baking from those in the central period.   There were thus available in the main series 14 hand and 14 machine-manipulated loaves each day.

Precautions were taken to eliminate certain possible sources of variation.   The correct absorption was determined by baking a series of loaves using different absorptions before beginning the main experiment.   The moisture content of the flour was determined each week and the water added adjusted to maintain a constant absorption.   The yeast was purchased on the same day each week and both the flour and yeast were kept in cold storage until required.   Previous to sampling for moisture determinations or for baking the flour was thoroughly mixed in a tumbling-box mixer.   The temperature and the relative hu-

midity in the baking laboratory were automatically controlled by an air conditioner at 30° C. ± 1° and 80% ± 2% respectively. The solutions of ingredients were made up and maintained at 30° C. while the yeast suspension was kept in a thermostatically controlled water-bath at 15° C. An automatic timing device was designed and attached to the Hobart-Swanson mixer and set to mix each dough for exactly 60 seconds.
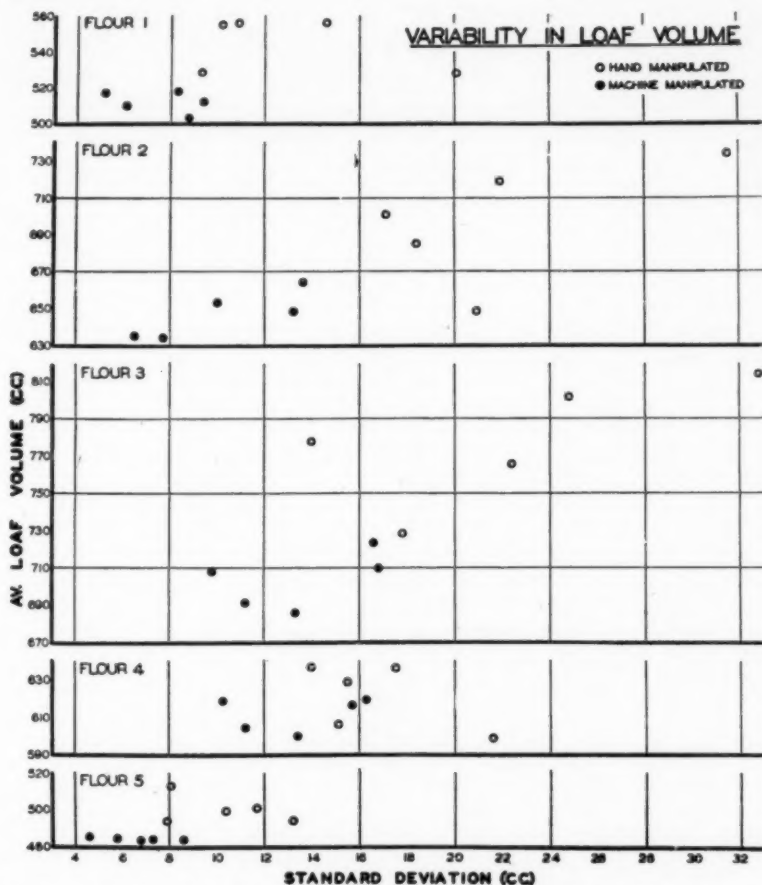

Fig. 2. Graph illustrating variability in loaf volume of hand and machine manipulated loaves.

Results

The main results of the experiment are shown in Figure 2, in which the average volume of the 14 loaves in each daily series is plotted against the standard deviation.

It will be seen that the variability within each series is definitely lower when the doughs are punched and moulded by machine. There

is some overlapping of the standard deviations by the two methods but there were only three days on which the variation was not less by machine than by hand. The values on these days were 9.3 and 9.4, 15.1 and 15.7, 8.1 and 8.6 for hand and machine manipulation respectively. The average coefficient of variability for hand manipulation was 2.6 and for machine manipulation 1.7. The variability of the 14 replicate loaves fluctuated considerably from week to week. For example, with flour 2 the standard deviation for hand manipulation varied from 17.1 to 31.5 and for machine manipulation from 6.5 to 13.6. This lack of stability of variance is one of the most unsatisfactory features of the present results. It will be noted, however, that the fluctuations are less pronounced when the machine is used and they appear in three of the five flours to be independent of the average loaf volume.

Similar fluctuation in the average loaf volume from day to day was found, but it will be readily seen that this fluctuation also is much less by machine than by hand. Machine manipulation gave lower loaf volumes than hand manipulation but the difference is not great. The relative differentiation between the samples is little affected by the lower loaf volumes given by the machine. Table I shows the average loaf volume of each flour calculated as a percentage of the average loaf volume for all five flours, the hand and machine results being treated separately. There is a remarkable correspondence between the two sets of percentages for the first three flours, and a fair correspondence for the others.

TABLE I

AVERAGE LOAF VOLUME OF EACH FLOUR AS PERCENTAGE OF AVERAGE VOLUME FOR ALL FLOURS

Flour	Hand	Machine
1	87.2	87.9
2	111.8	111.0
3	121.4	120.8
4	99.5	105.0
5	80.1	83.0

Discussion

In the light of these results it can be safely said that the use of the punching and moulding machines gives more accurate results than hand manipulation. While the volumes are lower when the machines are used, the differentiation between the samples is little affected.

It is necessary to examine the results obtained using the machines to determine whether their accuracy is sufficiently high. Baking tests are made to detect differences between samples of flour, and unless the variability of the method is low, small differences can only be detected

with certainty by extensive replication. In order to be absolutely sure
that a difference of 20 cc. between two means of duplicates is not due
merely to chance variation it is necessary to reduce the standard devia-
tion within days to a maximum of 8 cc. and the additional deviation
between days to a maximum of 3 cc. Of course a reduction in the
variability within days would make allowable a greater variation be-
tween days and vice versa. The results of this experiment do not come
within these limits but there are indications that with further refinements
it may be possible to achieve this accuracy. Eight of the 25 bakings
using the machine gave standard deviations of less than 8 cc. within
days. While standard deviations between days could not be accurately
calculated from the available data, certainly one and possibly two (flour
1 and flour 5) of the flours appear to be consistent enough to give the
desired results.

NOTES ON INTERPRETATION OF STANDARD BAKING TEST [1]

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(Read at the annual meeting, June, 1934)

Since 1925, much work has been done on the standardization of
baking technic of the official A. A. C. C. bread baking test by various
committees under the leadership of Blish (1928), Harrel (1929), Bailey
(1930, 1931), Coleman (1932) and Geddes (1933). The work of
Canadian investigators, notably that directed by Geddes and Larmour,
contributed much to the proper evaluation of the variables which enter
into the baking test.

At present, persons using this test can proceed with a reasonable
feeling of confidence in the results obtained. Chemists fully informed
as to the variables will regard the results of the test in the proper pro-
portion. There will always be differences in loaf volume, with the
same flour, as obtained by various workers even in the same laboratory,
due to individual technic in those parts of the test where the personal
equation enters, but the difference will be found consistent. Loaf
characteristics are essentially the same. It is evident that it does not
make a flour any better if one operator obtains five percent more volume
in his test loaf than another worker in the same or different laboratory.
It is the other results of the test and the proper interpretation that
count. This has been recognized by the chairmen of the various Baking

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[1] Subcommittee report, 1933–34 Committee on the Standardization of Laboratory Baking.

Test Committees and others interested in the standardization of the baking test by cereal chemists. Geddes in reporting the activities of his committee in 1933 speaks of the necessity of interpreting the test.

Method of Reporting Results

While the ground has been broken for the scientific reporting of the results of the official baking test by Blish (1928a), there are but few laboratories that have taken up the suggestions and applied them in reporting their results of the test, and yet some common method of recording the results must be used before a mutual understanding of results can be arrived at. For permanent record of results obtained, the method employed by Blish, of assigning a letter to the type loaf obtained, is of much value.

Some investigators have suggested a single numerical value for the results of the test. As Platt (1933) states, " Single figure baking scores are very useful and save much time and mental effort, but in cereal chemistry they must be used with a tremendous mental reservation."

We are hardly justified in assigning a numerical value to the result of the baking test any more than giving the results of an oil analysis a single score, or lumping together the findings of a steel analysis. An oil chemist reviewing the laboratory results of a lubricating oil can describe the field from which it came, how it has been treated, and for what purpose it can be used to best advantage. The results of a baking test may be expressed in terms of :

> Absorption
> Loaf volume
> Loaf type and outside appearance
> Crust color
> Grain and texture
> Crumb color
> Reaction to mixing
> Reaction to bromate

Absorption

The percentage absorption of a flour is important to the baker, as it affects the yield of bread obtained per barrel of flour. He is interested in knowing the relative absorption of flours that come into his bakery. If the laboratory has properly explained the significance of change in apparent absorption due to change in moisture content during storage, the baker will not belittle the absorption as reported on a standard moisture content, for this is practically standard for a given brand of flour over a period of a crop year.

There is a definite relationship between laboratory absorption and shop absorption when allowance is made for moisture losses during storage. It is obvious that it would be impossible to attempt to bring the two absorptions into harmony, as the formulas used in commercial baking vary so widely in water absorption, depending on the kind of bread being made. For example, in one case the same flour was being used in one shop satisfactorily with an absorption of 54% and in another with 62%. This was due primarily to the difference in type of bread being produced.

Portions of several hundred cars of one brand of flour were baked in the laboratory with the same absorption during the course of a crop year. Further, there is very little difference between the absorption of a certain class of flour; i.e., a series of six straight grade hard winter wheat flours, having the same protein content during this crop year, has shown a maximum variation of 1% absorption.

There should be a greater harmony between laboratories on absorptions. The use of the standard baking test as revised will do much to help this. Collaborative work on several classes of flour will be of interest. An official definition of what constitutes absorption should be described by the A. A. C. C.

Volume

The fact that this is the only result of the baking test that can be expressed in numerical terms has caused undue emphasis to be placed upon it. The size of the loaf is the result of several factors, such as physical condition of the gluten aggregate and rate of gas evolution, and the effect of these must be taken into consideration when interpreting the figures representing loaf volume. A large volume does not necessarily indicate superior baking strength, but may really be an indication of weakness in the gluten or excessive gas production. Volume cannot be considered alone; the type loaf and internal characteristics enter into the interpretation of the results for evaluation for commercial purposes. Rich (1934) notes this in a recent article in Cereal Chemistry when speaking of chemical changes in wheat flour by artificial maturing agents. He says, " In a study of this kind loaf volume alone would in many cases lead to erroneous conclusions." No doubt, for the cereal chemist interested in evaluating wheat types the volume of the test loaf is of considerable value, but in the commercial testing of flour in the baking laboratory it is of secondary importance. It must be used with tremendous reservation. Such a laboratory may be testing spring wheat, soft and hard winter wheat flours, bleached and unbleached, matured or unmatured, which give a series of loaf volumes far out of

proportion to the protein content, due to the physical condition of the gluten. Such a laboratory often obtains smaller volumes from the high protein flours than from one with less protein but more matured. Yet each of the flours fills a certain need in baking practice and under certain conditions will give entirely satisfactory results. Probably this is what Kent-Jones (1934) had in mind when he wrote: " The volume of the ' pup ' loaves is, of course, the principal criterion and is, in my opinion, an unfortunate one."

We cannot hope to correlate loaf volume of the test loaf with that obtained in commercial practice, unless we consider the formula and shop practice employed. If no oxidizing agent, such as potassium bromate, is being used in the formula, then the volume of the commercial loaf follows that of the test loaf provided the flour is handled as the other properties of the test loaf indicate it should be. This means that the mixing period of the basic loaf must be adjusted to the mixing requirements of the flour, otherwise it will be found that the volume of the commercial loaf is not in proportion to that of the basic loaf.

Tests made in the laboratory using a commercial bread formula and the sponge method of bread production demonstrate that while, as to be expected, the volume of the commercial loaf is much greater than that of the basic test loaf, the same relative difference between flours is exhibited. This is true of commercial bake-shop production and answers any criticism that may arise as to the utility of the " pup " test.

The obvious correlation of loaf volume with protein content may be of interest but is of little commercial value unless the same type wheat is used in milling the flour and the maturation of the gluten is the same. A flour of 11.50% protein content, that yields a basic loaf of 540 cc. volume, may be far better for certain purposes than another of 13.00% protein which makes a basic loaf of only 510 cc. On the other hand, of two flours of the same protein content, the one giving 500 cc. basic loaf volume may be better suited for some bakeries than the other which gives 540 cc. loaf volume. The determining factor will be the type loaf and grain and texture characteristics.

If protein content was the only factor determining the baking characteristics of a flour, there would be little need for a baking chemist. The policing of flour for ash and protein content would not require a trained cereal chemist.

If loaf volume, whether that of the basic or stimulated loaf, was the determining factor, the task of the baking chemist would indeed be a simple one. But such is not the case. Flour quality cannot be determined from loaf volume alone.

The volume of the stimulated loaf containing bromate, is not in itself of any more value than the basic loaf. Used in conjunction with the

basic loaf and taking into consideration the change in grain and texture that may occur, it gives valuable information. And yet, a man experienced with the test can predict from the results of the basic test just about how the bromate loaf will look. It is this ability to interpret the results of the standard test which cereal chemists must acquire. We must develop the ability to interpret the results we obtain.

Loaf Type and Outside Appearance

As stated before, the method suggested by Blish, of assigning a letter to the type loaf obtained, is of much value. Loaf types are reproducible within one laboratory. Another laboratory may obtain a slightly different picture but certain outstanding characteristics will predominate, which will permit of the same interpretation in the end. It is significant whether a flour gives a smooth or ragged break in the top of the loaf. It is of interest to know whether the base of the loaf is smooth or of varying degrees of roughness. The outside appearance of the basic test loaf is not correlated with the outside appearance of the commercial loaf and it would be incorrect to imply that it is, but it reveals gluten characteristics which, taken in connection with the other results of the test, particularly loaf volume, grain and texture, and crust color, enables one familiar with the test to accurately predict how the flour will react under certain conditions.

The base of the test loaf may be rough and the top show a very ragged break indicating gluten in a highly oxidized condition, but the same flour properly handled as to formula and procedure will not reveal the same external appearance in the commercial loaf. This is because of the drastic treatment which the basic test method gives a flour. The cereal chemist must carefully watch loaf types if he expects to fully appreciate the inherent qualities of a flour. The reason many disagreements occur between chemists is because the particular baking test they use does not fully reveal the differences in loaf types and external appearance.

Crust Color

The importance of crust color and its relation to diastatic capacity of a flour has been given much consideration by various investigators, chiefly Blish (1929) and Moen (1930). While the chemical means of determining this give numerical figures of much information and interest, it is an additional test which in the ordinary control testing of flour is not necessary if a baking test is made. The A. A. C. C. baking test will give ample information regarding the gassing power of a flour for commercial work. Basic test loaves which show a medium crust

color give satisfactory results in commercial sponge dough fermentation as now practiced. This is demonstrated in the satisfactory increase in sponge temperature which accompanies normal gas evolution. Tests made of the rate of gas evolution from commercial sponges verified this conclusion. Curves showing the rate of gas evolution from flour which had not been given special diastatic treatment and one which had were obtained by taking a piece of the sponge when mixed and placing it in a bottle attached to a gas measuring system, the bottle having previously been brought to the same temperature as the sponge. This bottle was then placed in and entirely surrounded by the sponge. Thus the actual conditions of sponge fermentation were utilized. The rate of gas evolution increased with increase in temperature. The effect of the lack of proper diastase was clearly shown in the drop in rate of gas evolution and smaller increase in temperature. A flour lacking in proper gassing strength will give a pale, shell-top loaf by A. A. C. C. test method. Thus, the baking chemist who finds a flour to give a normal crust color can be confident it will be satisfactory for all normal fermentation requirements. The baker will find a normal increase in sponge temperature and be satisfied with the behavior of the sponge.

Grain and Texture

Some attempts have been made in the past and are still being made to properly describe and classify grain and texture. It is a difficult problem because of the many slight variations that can arise. I have found it possible to use the terms spherical, elongated, and attenuated, to describe the grain of the test loaf. These were introduced by E. E. Werner, the founder of this test, and by Harry Weaver, our first president. They present a picture to one who is accustomed to them which enables a proper interpretation of the usefulness of a flour. Grain of the basic test loaf is correlated with that in the commercial loaf, provided no special material, such as yeast food, is used, or special treatment, such as twisting, is given the dough. Werner and Herman (1928) have fully described the significance of grain and texture characteristics. A laboratory servicing the baking industry must be very observant of grain and texture. These qualities are more important than volume.

Crumb Color

The crumb color of the basic loaf is correlated with that of the commercial loaf made without any special bleaching materials. The bakery chemist can accurately predict crumb color in the commercial loaf from the results of the basic test loaf.

Reaction to Mixing

One hears a great deal about the mechanical development of bread dough. The way the flour holds up under excessive mechanical treatment is of great importance to the baking chemist. He must be able to tell whether one flour will require more mixing than another and wherein they differ in their ability to stand severe mechanical abuse. For this purpose, a machine like the Brabender Farinograph could be used to advantage. The supplementary A. A. C. C. test in which the mixing time is varied, can be used, but a clear understanding of the results must be had. How long should a test dough be mixed to get a picture of the way the flour will behave in the shop? The basic procedure calls for one minute. This is not enough for every flour, although I must state I am referring to 200-gram doughs. The chemist, by watching the dough as it mixes, can catch the breaking point within a few seconds. Further mixing is undoubtedly bringing another factor into the picture. With the Hobart-Swanson mixer, development is extremely rapid. In 3 minutes the dough may be completely broken down, in some cases 4 minutes is required, but not many flours will stand 5 minutes. The average flour at the end of that time is a stickly, stringy mass of dough which is almost impossible to handle. The average baker would call such a dough way over-mixed and he would be correct. Nothing is to be gained by mixing to that degree in the bakery. In other words, the mixing need not be carried over 5 minutes using 200 grams of flour in a Hobart-Swanson mixer. Further mixing might be interesting but is of little value. To put it another way, 1½ minutes mixing of a dough in the Hobart-Swanson is equivalent to the first 8 or 10 minutes in a commercial mixer (sponge dough process), the variation in time being due to the difference in mixers. After that, 1 minute is equivalent to about 4 minutes in the commercial shop, so that it can readily be seen that 5 minutes is more than enough.

The chemist must have the ability to interpret the basic loaf and to observe differences in flour during mixing. The chemist experienced with the A. A. C. C. baking test will know that any flour which stands 5 minutes in the Hobart-Swanson mixer will withstand any mechanical development it can possibly be given in commercial mixing.

Reaction to Bromate

As I said before, the reaction to bromate can be in many cases accurately predicted from the general characteristics of the basic loaf by one acquainted with the test.

This reaction is of great importance to those bakers who are using yeast food containing oxidizing agents of the bromate type. The baking

test properly interpreted will tell him how much of this material he can safely use. It will also give a general idea of the gluten characteristics of the flour which can be interpreted in the shop in terms of grain and texture. It will tell how much a flour has been matured.

The analytical results of a flour analysis afford an excellent means of classifying flours in a general way and of checking uniformity, but the baker, who after all is the final judge of a flour, is interested in the baking qualities as they fit in with his shop procedure which is probably typical of average shop practice throughout the country. Here again, a comparison can be made with a steel analysis. A chemical analysis of steel will classify it and be a check on uniformity, but the heat treatment it has had must be known if its properties and usefulness for a certain purpose are to be utilized.

As cereal chemists, we must give more attention to the interpretation of the results of our work. It is the complexity of this problem which makes cereal chemistry so interesting.

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A STUDY OF TEMPERATURE CHANGES IN A SMALL LABORATORY OVEN WHEN DRYING FLOUR BY THE 130° C. OVEN METHOD [1]

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Introduction

When making moisture determinations by the 130° C. oven method, it was noticed that during the drying of the samples, considerable differences of temperature existed in various parts of the oven. This difference was particularly noticeable between points above and below the oven shelf. There was also considerable variation in temperature at the beginning of the drying period which made it difficult to meet the A. A. C. C. specifications of 130° C. ± 3° C.

As a result of these observations, some work was done to find out how the changing of various conditions would affect the moisture results and also to follow as nearly as possible the temperature changes taking place in the oven.

In order to have a standard of comparison, determinations were made from time to time by the vacuum method.

Experimental

The air oven used was of the small laboratory type. When measured on the inside it was found to be approximately 30½ cm. wide, 33 cm. deep, and 35½ cm. high. Several shelves were provided. It was equipped with two 500-watt intermittent heater units, both of which were used simultaneously in these experiments. The thermostat control was composed of a lever arrangement actuated by the expansion or contraction of a rod located in the rear of the oven compartment. Since this rod extended from the top of the compartment to within about an inch of the heating element, it was actuated by temperature changes, both above and below the shelves. Three holes were provided on each side for ventilation. These were spaced between the lower and top part of the compartment.

For these experiments, only one shelf was used and it was placed almost midway between the top of the compartment and the upper

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surface of the heating unit. The shelf had square perforations, through which the air could circulate. In order to avoid undue cooling of the oven when placing the samples in it, they were first placed on a small aluminum tray which was then quickly inserted in it. The perforations in this tray were also in the form of small squares of the same size as those of the shelf. By a little manipulation, the tray could be so placed that its perforations matched those of the oven shelf and consequently did not unduly obstruct air circulation.

Three thermometers were placed in the oven. One of them was inserted through the top of the oven so that the tip of its bulb was 3 cm. above the shelf. The others were placed through side openings so that the bulb of one was 11 cm. above the shelf, and the bulb of the other 1 cm. below the shelf. These last two thermometers were inserted about 13 cm. into the oven. The thermometers will hereafter be referred to in the order they were described as A, B, and C.

The official vacuum method specifies that the samples shall be dried to constant weight (approximately 5 hours) at a partial pressure equal to one inch of mercury. The lowest partial pressure obtainable with the available equipment was from two to three inches, the pressure being read from a gauge on the oven. To partially compensate for the error so introduced, the samples were dried for 6 hours in loosely covered dishes with slip-in covers. When releasing the vacuum, the air was drawn in through a calcium chloride drying tube.

Determinations were made with three patent flours, namely, a flour milled chiefly from hard spring wheat, another from soft wheat, and a third from hard winter wheat. Each of these was put in a glass bottle and a rubber stopper inserted to insure against any change in moisture. Each series of closely related determinations was made on the same day to avoid any daily changing conditions which might affect the results. This precaution was taken since Spencer [2] had shown that the moisture results appeared to be affected by changes of relative humidity. The number of determinations made were so chosen that the total number of samples weighed would be even multiples of three. This allowed an equal number of samples to be weighed from each of the flours. Each series of determinations was made twice and only the averaged results are shown in the report.

Before beginning the determinations, the empty oven with the shelf in place was adjusted so that it would maintain a temperature of 130° C. ± 2° C. according to thermometer A. After the oven was found to be operating within the limits specified, the temperature of thermometer A was watched until it read 130° C. The readings of all three ther-

[2] Spencer, C. G. 1925. The quantitative determination of moisture in wheat flour. J. Assoc. Official Agr. Chem. 8: 301–311.

mometers were then recorded and the samples put in the oven as quickly as possible. The lowest temperature readings attained by the thermometers and the approximate time to reach them were recorded. The rate at which the oven regained its temperature was determined by reading the thermometers at ten minute intervals throughout the determination, the last reading being taken a little early so that the oven could be opened in exactly one hour. The covers were quickly slipped on the dishes before removing any of them from the oven. They were then placed in desiccators to cool.

Before making any moisture determinations, an experiment was made to determine how much the oven was cooled and how rapidly it recovered to its original temperature when only the tray and nine empty dishes were placed in it. To do this, the empty oven was first set to operate at 130° C. ± 2° C. according to thermometer A. The temperature readings of the three thermometers were taken and the tray, moisture dishes, and covers were put in. Except for the minimum temperature reading, the thermometers were read at ten minute intervals. The data are shown in Table I and Figure 1.
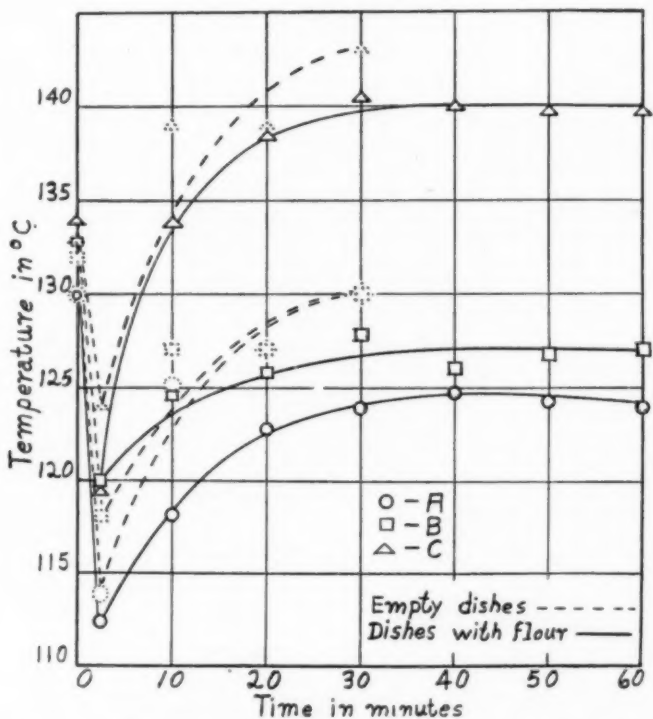

Fig. 1. Curves showing temperature changes in oven when loaded with flour samples and when loaded with empty dishes.

TABLE I
TEMPERATURE OF OVEN WITH TRAY AND EMPTY DISHES

	Thermometer		
	A	B	C
	°C.	°C.	°C.
Empty	130	132	133
Minimum after loading	114	118	124
10 minutes " "	125	127	139
20 " " "	127	127	139
30 " " "	130	130	143

The first series of moisture determinations was made to observe how the temperature and apparent moisture percentages were affected by the number of samples in the oven.   Experiments were made in which groups of 6, 9, 12, and 15 two-gram samples were dried.   The results are shown in Tables II and III.   The vacuum method results shown in Table III and in certain subsequent tables are the average of five determinations made on different days and represent a standard of comparison for the other findings.

TABLE II
EFFECT UPON OVEN TEMPERATURE OF DIFFERENT SAMPLE LOADS

Number of samples	6			9			12			15		
Thermometer	A	B	C	A	B	C	A	B	C	A	B	C
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Empty	130	133	134	130	133	134	130	133	134	130	132	134
Minimum after loading	115	121	120	113	120	118	111	119	121	111	120	119
10 minutes " "	120	126	133	119	125	136	117	123	134	117	125	132
20 " " "	124	125	138	125	127	139	121	125	138	121	126	138
30 " " "	125	128	141	125	129	140	124	127	142	122	127	139
40 " " "	126	123	141	125	127	139	125	126	141	123	128	139
50 " " "	125	127	140	125	127	139	124	125	140	123	128	140
60 " " "	125	127	140	125	129	140	124	126	140	122	126	139

TABLE III
EFFECT UPON MOISTURE RESULTS OF DIFFERENT SAMPLE LOADS

Number of samples	Soft wheat flour	Hard spring wheat flour	Hard winter wheat flour
130° C. oven method			
	%	%	%
6	12.36	12.09	12.77
9	12.36	12.09	12.70
12	12.30	12.04	12.73
15	12.23	12.00	12.70
Vacuum method results			
	12.52	12.26	12.99

Usually about 2½ minutes were required for the thermometers to reach the minimum temperature point and again begin to rise. The thermostat usually made contact for about 4 minutes after the samples were put in the oven. It then remained open about 4 to 6 minutes. Thereafter its average contact period was about 1½ minutes, and its open period about 3¾ minutes.

The temperature changes in the oven during each of these determinations were so nearly alike that all the temperature data of Table II were averaged together. The results so obtained are shown in Table IV and Figure 1.

TABLE IV

VARIATION OF OVEN TEMPERATURES DURING THE DRYING OF SAMPLES

	Thermometer		
	A	B	C
	° C.	° C.	° C.
Empty	130.0	132.8	134.0
Minimum after loading	112.5	120.0	119.5
10 minutes " "	118.3	124.8	133.8
20 " " "	122.8	125.8	138.3
30 " " "	124.0	127.8	140.5
40 " " "	124.8	126.0	140.0
50 " " "	124.3	126.8	139.8
60 " " "	124.0	127.0	139.8

In addition to the series of determinations just described, two sets of nine samples of two grams each were dried for 30 and 90 minutes, respectively. The results of these dryings and also the result of the 60-minute period of Table III are shown in Table V. Since the temperature data contributed nothing that was not already shown, they were omitted.

TABLE V

EFFECT UPON MOISTURE RESULTS OF DIFFERENT LENGTHS OF DRYING TIME

Drying time	Soft wheat flour	Hard spring wheat flour	Hard winter wheat flour
130° C. oven method			
	%	%	%
30 minutes	12.31	12.01	12.58
60 "	12.36	12.09	12.70
90 "	12.38	12.10	12.72
Vacuum method results			
	12.52	12.26	12.99

To observe how large differences of temperature affected the results, another series of determinations was made. The oven when empty was

adjusted to operate at 110° C. ± 2° C. according to thermometer A. After recording the temperatures of the three thermometers, a load of nine samples of two grams each was placed in the oven. The temperature changes were then noted as in the previous experiments. This procedure was repeated for temperatures of 120° C., 130° C., and 140° C. The results are shown in Tables VI and VII, and in Figure 2.
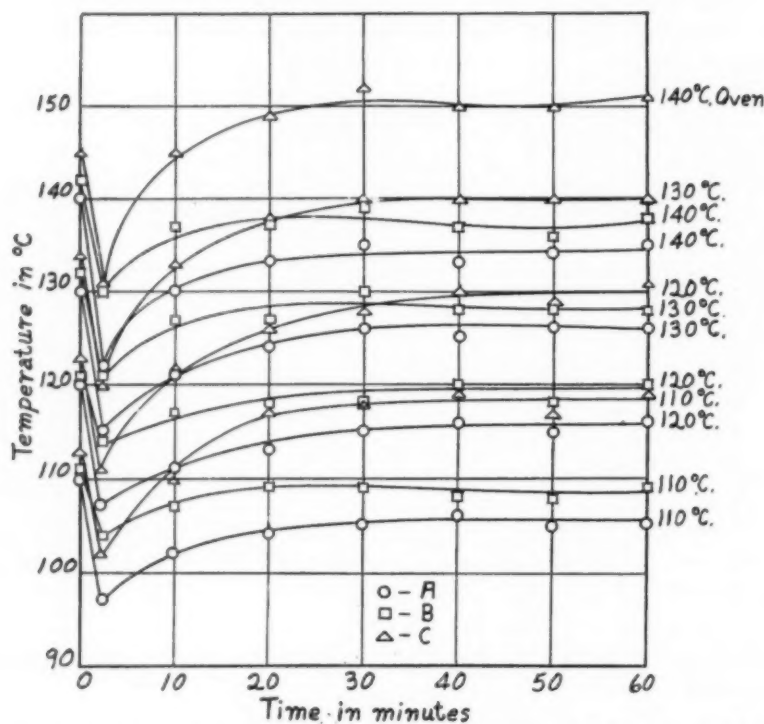

Fig. 2. Curves showing effect of flour samples upon oven temperatures at different oven adjustments.

TABLE VI

Effect upon Oven Temperatures at Different Oven Adjustments

Oven adjusted to	110° C.			120° C.			130° C.			140° C.		
Thermometer	A	B	C	A	B	C	A	B	C	A	B	C
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Empty	110	111	113	120	121	123	130	132	134	140	142	145
Minimum after loading	97	104	102	107	114	111	115	121	120	122	130	131
10 minutes " " "	102	107	110	111	117	122	121	127	133	130	137	145
20 " " "	104	109	117	113	118	126	124	127	138	133	137	149
30 " " "	105	109	118	115	118	128	126	130	140	135	139	152
40 " " "	106	108	119	116	120	130	125	128	140	133	137	150
50 " " "	105	108	117	115	118	129	126	128	140	134	136	150
60 " " "	105	109	119	116	120	131	126	128	140	135	138	151

TABLE VII

EFFECT UPON MOISTURE RESULTS AT DIFFERENT OVEN ADJUSTMENTS

Oven adjusted to	Soft wheat flour	Hard spring wheat flour	Hard winter wheat flour
	130° C. oven method		
° C.	%	%	%
110	11.97	11.66	12.33
120	12.19	11.79	12.47
130	12.34	12.06	12.69
140	12.44	12.15	12.81
	Vacuum method results		
	12.52	12.26	12.99

Discussion and Conclusions

The temperature curves in Figure 1 show that there is a considerable difference of temperature at various locations in the oven. The greatest difference appears between points directly beneath the samples and directly above them. It would appear that this difference is due chiefly to the obstruction of the free circulation of air by the shelf and the sample dishes upon it. In support of this conclusion, we find that the differences in temperature as registered by thermometers A and B were quite large whether the dishes were empty or contained flour.

It is apparent from the curves of Figure 1 that when a number of flour samples were placed in the oven, the initial temperature for which the thermostat was adjusted was never reached by the thermometers above the flour, but after a considerable time lag they appeared to reach a more or less constant value. The extent of this time lag was not due to insufficient heating capacity since, as stated in the experimental section, the thermostat made and broke contact at frequent intervals. The slow rate of temperature rise seemed rather to be due to the fact that the thermostat was responding to a mean value of the temperatures above and below the shelf. When electrical contact was made, it seemed that a considerable quantity of heat quickly accumulated below the shelf, and since it acted on approximately half of the thermostat expansion rod, it was sufficient to open the electrical circuit even though the space above the shelf was considerably below 130° C. as indicated by thermometer A.

It is possible that this condition could have been partly, if not entirely, remedied by placing the shelf very near the floor of the oven. However, this would cause a great concentration of heat directly beneath the samples which may or may not be desirable. If the 130° C. temperature is desirable only above the shelf, the difficulty might be

remedied by designing the thermostat so that its operating element was entirely in the vicinity of the flour samples.

This discussion brings out two points which it is believed should be mentioned in the method specifications.   They are (1) the placement of the thermometer, *i.e.*, how far it should be from the oven shelf on which the samples are placed and whether it should be above or below the shelf, and (2) the time allowable for the oven to regain its original operating temperature after the samples have been inserted.   The oven as used in these experiments did not regain its original operating temperature within the one hour period, according to the thermometer directly above the flour samples.   However, as before stated, by a different arrangement of the shelf or thermostat element this undesirable feature might be minimized.

The moisture results in Table III show that considerable variation in the number of samples being dried may be tolerated without greatly affecting the results.   The fifteen samples that were dried were about all that could readily be placed on the single oven shelf and the results appeared to be affected by only about 0.1%.   However, since a difference in apparent moisture results does appear to exist due to oven load, it would seem advisable to specify in the method the maximum and the minimum numbers of two gram samples to be dried per unit of oven space.

Considerable variation in drying time can apparently be tolerated without greatly affecting the results, but changing the oven temperature affects them markedly as may be seen from Table VII.   Very little difference could be seen in the moisture results obtained from the three flours, *i.e.*, it appears that all three flours behaved in a similar manner during the drying periods and yielded results of similar trend.

The vacuum method as used in these experiments gave moisture results higher than the 130° C. oven method.   It might be reasoned that this was due to the fact that the temperature of the oven above the shelf and in the immediate vicinity of the flour was below 130° C. during the drying period.   This may partly account for the low results, but it will be noticed that even when the oven was set at 140° C., the moisture results were also lower than those obtained with samples dried in the vacuum oven.   The temperature curves for the 140° C. oven of Figure 2 show that during most of the drying period all the thermometers were registering temperatures above 130° C.

The official vacuum method states that the dishes are to be loosely covered during the drying period and when the vacuum is released the air before entering the chamber is to be desiccated.   Spencer [2] showed that higher and less erratic results were obtained by the vacuum method when the dishes were loosely covered during the drying process than if

the covers were completely removed. It would seem that this difference could only be due to the reabsorption of moisture before the covers could be put in place. In the 130° C. oven method the samples are dried in uncovered dishes and there is no means of preventing reabsorption of moisture while the dishes are being covered.

The difference in moisture results obtained by the two methods may have been due to the dried samples taking up moisture from the atmosphere before the covers could be put on the dishes. Since these experiments were made in a laboratory located near the seacoast, it is quite probable that the relative humidity is normally higher than farther inland. This might cause the dried samples to pick up an abnormal amount of moisture and so show lower comparative results than other observers have found.

Shuey [3] in a report of moisture methods also found after collecting the collaborators' results that the samples dried in the 130° C. oven gave lower moisture results than those dried by the official vacuum method.

Summary

Thermometers placed in the oven registered different temperatures in various parts of the drying chamber.

It was found that from 6 to 15 flour samples of two grams could be dried at one time in an oven, the internal measurements of which were 30½ x 33 x 35 cm., without affecting the moisture results by much more than 0.1%.

Drying samples for 30, 60, and 90 minutes affected the results by about 0.1%.

Oven temperature between 110° C. and 140° C. considerably affected the results.

The specifications for the 130° C. oven method should definitely fix the position of the bulb of the thermometer in relation to the samples and the amount of time to be allowed for the oven after loading to recover its original temperature. The maximum and minimum number of samples to be dried at one time should also be specified.

[3] Shuey, G. A. 1925. Collaborative study of moisture methods. Cereal Chem. 2: 318–323.

OBSERVATIONS ON GRAIN AND TEXTURE

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(Presented at the Annual Meeting, June, 1934)

According to the American Institute of Baking's bread score card, which is widely used in the baking industry, the crumb structure is responsible for 25% of the total score of 100 assigned to an ideal loaf of bread. Grain, or the visual appearance of the crumb is given 10 points, while texture, or the feel of the crumb, is given 15 points. Obviously, in this system of scoring, crumb structure plays a very important part in determining the quality of a loaf of bread.

Most bakers and cereal chemists know that crumb structure in bread made from high quality flour can be varied considerably by changes in the method of production. The primary interest of the cereal chemist, however, is to know what part the flour plays in producing satisfactory grain and texture in commercial practice. This problem of grain and texture constitutes the basis for this paper.

The A. A. C. C. baking method or some modification of it is being used in many laboratories. The modifications in some instances consist of changes in the formula, such as the addition of shortening, oxidizing agents, malt, etc., and changes in the method, such as temperature, mixing, or fermentation time. Although deviations from the standard procedure may be numerous, most laboratories using the "pup" loaf method also use what is considered a lean formula. The question then arises: Are significant differences in grain and texture obtained by the A. A. C. C. baking method or slight modifications thereof, also noticeable in commercial practice?

As a matter of explanation, the writers opinion of a "significant difference" is the smallest difference in grain and texture between any two flours which can be consistently duplicated. A "significant difference" is not necessarily small. In fact, it may be quite large, depending to some extent at least, upon the efficiency of the operator. Uncontrollable influences also play an important part in actual cell formation of a loaf of bread.

A good illustration of the magnitude of variations in grain and texture which occasionally occurs in this laboratory is shown in Figure 1. Both loaves were made by the same method, from the same flour, and on the same day. Had these two loaves been baked from different flours
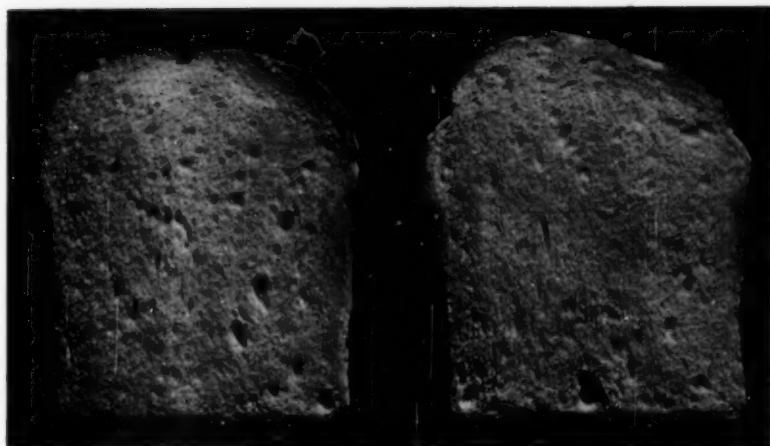
Figure 1.

and taken at face value, it is very probable that the flour represented by
the right hand loaf would have been scored substantially higher for grain
than the loaf on the left.   Incidentally, the right hand loaf would have
also been scored higher for color, due to the effect of the elongated cell
structure upon crumb color.   Obviously, an operator must know the
magnitude of the variations he is likely to have between replicates before
he is in a position to record only significant differences in his reports.
Recording insignificant differences only leads to confusion and lack of
confidence in baking reports.

Figure 2 shows loaves made from two experimentally milled flours.
The A. A. C. C. formula plus 2% shortening was used and doughs were

Figure 2.

given 3 hours fermentation and 55 minutes proof at 27.8° C., instead of 30° C., called for in the standard method. The difference in crumb structure between these two loaves is very significant by this method of baking. Approximately this same difference was replicated many times.

Figure 3 shows results from the same two flours referred to in Figure 2, but baked into one pound loaves with a formula containing 2% yeast, 2% salt, 4% sugar, 4% dry skim-milk, ¼% yeast food, and 2% shortening. Fermentation time was three hours at 27.8° C. and doughs were proofed to the same volume at 35° C. In Figure 2 the right hand loaf is substantially inferior to the left hand loaf. In Figure 3 the difference in grain and texture shown in Figure 2 is completely wiped out.

Figure 4 shows results obtained from flours A, B, and C. All doughs were fermented and proofed at 30° C. The left hand vertical
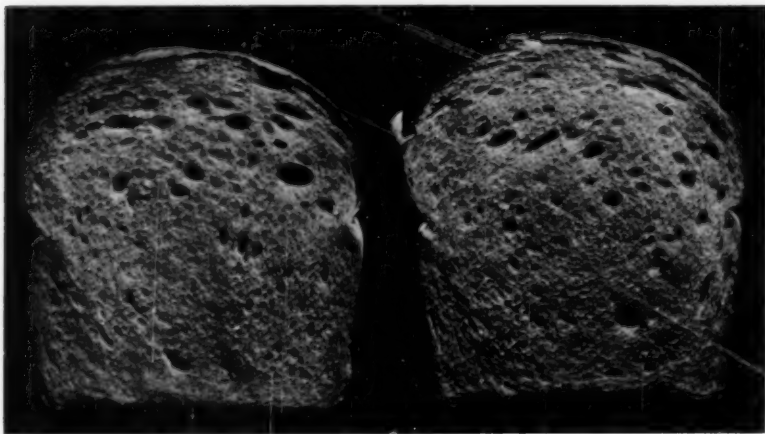

Figure 3.

row shows loaves from flour A baked by three different methods. Likewise the middle and right hand rows show loaves from flours B and C, respectively.

The loaves shown in the top horizontal row, numbers 1, 2, and 3. were baked by the standard A. A. C. C. method. It will be noted that a large difference in crumb structure is obtained from the three samples by this method, and that flour A is substantially superior to flours B and C.

Loaves 4, 5, and 6 were baked by a modification of the A. A. C. C. standard method which consisted in the addition of 2% shortening and proofing doughs to same volume. Loaves numbered 4 and 5 required 50 minutes proof, and 6 required only 40 minutes proof to bring doughs to a point slightly over top of pan. It will be noted that all three loaves are superior to those in the top row, but flour A still has a crumb struc-

ture superior to that of flours B and C.   The loaf from flour C showed a decided improvement, due to the addition of 2% shortening and proofing to definite volume.

Loaves 7, 8, and 9 were made with a commercial formula containing 2% yeast, 2% salt, 4% dry skim-milk, 4% sugar, ¼% yeast food, and
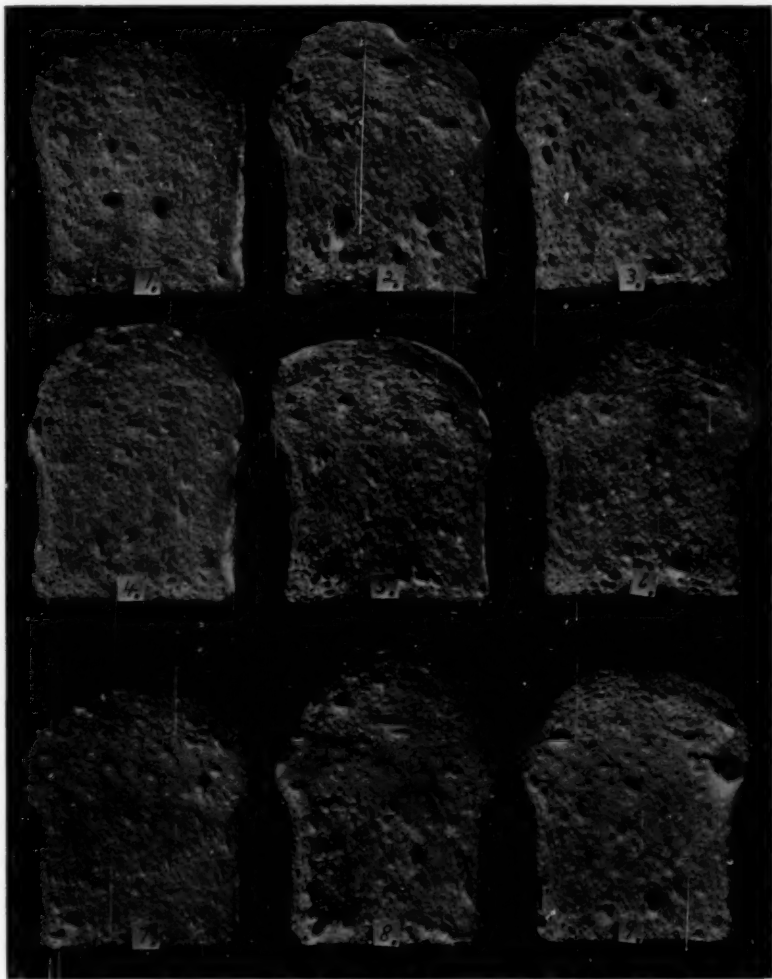

Figure 4.

2% shortening.   Fermentation time was 3 hours and all doughs were proofed to same volume.   Loaf number 7 required 45 minutes proof while loaves numbered 8 and 9 required 40 minutes proof to bring doughs to a point slightly above top of pan.   These loaves indicated that all three flours produce very good grain by this method of baking and

differences indicated in results obtained by the other two baking methods are practically wiped out.

The cereal chemist often wishes to know the effect certain yeast foods, bread improvers, malt, etc., have upon the crumb structure of his flours in commercial practice. Can he determine this by his laboratory method of testing?
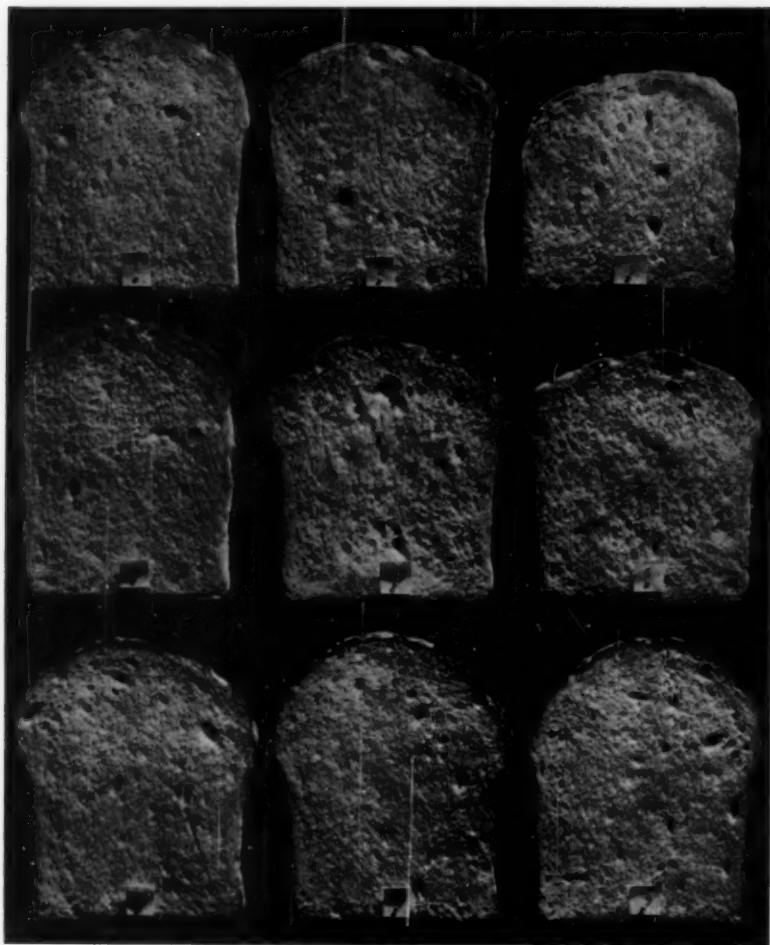

Figure 5.

Figure 5 shows nine loaves all made from the same commercially milled flour. Doughs were fermented and proofed at 30° C. The left hand vertical row, or loaves numbered 1, 4, and 7, contained no yeast food. Loaves 2, 5, and 8 contained ½% yeast food, while loaves 3, 6, and 9 contained 1% yeast food.

The top horizontal row, or loaves numbered 1, 2, and 3, was baked by the A. A. C. C. standard method except that all loaves were proofed to the same volume or to a point slightly above top of pans. Loaf 1 required 50 minutes proof, while loaves 2 and 3 required 40 and 30 minutes, respectively. Note the open grain in loaves 2 and 3, due to yeast food.

Loaves numbered 4, 5, and 6 were baked by the A. A. C. C. standard method which calls for 55 minutes proof. By this method loaf 5 proofed much higher than loaf 4, and loaf 6 proofed higher than loaf 5. Note that all three loaves are more open than loaves 1, 2, and 3 and that there is a decided openness in crumb structure in loaves 5 and 6, due to the yeast food.

Loaves numbered 7, 8, and 9 were baked by a commercial formula containing 2% yeast, 2% salt, 4% dry skim-milk, 4% sugar, and 2% shortening. Doughs were proofed to a uniform height or slightly above top of pan. Note that yeast food had practically no effect upon crumb structure.

Summary

Various flours were baked by a number of methods more or less popular in laboratory test baking. The crumb structures of these loaves were compared against loaves made with an average commercial formula.

Results indicate that the formula and method of baking had a very large influence upon the crumb structure obtained. The A. A. C. C. standard method and various modifications of this method, used in this work, were without doubt very efficient in detecting deficiencies or variations in crumb structure inherent in the flour, but fairly large variations between flours observed by these methods were often completely wiped out when the same flours were baked by commercial formulas.

The question now arises: Should the cereal chemist take the variations in grain and texture, which he finds to be significant, by these laboratory methods seriously? If so, is he in danger of penalizing many flours which in reality perform very satisfactorily in commercial practice?

These observations suggest the desirability of studies arranged for the purpose of answering these questions and determining the relationship of grain and texture obtained by lean formulas used in many laboratories to results of the same flours baked by average commercial formulas and bakeshop methods.

REPORT OF THE CEREAL SECTION OF THE ASSOCIA-
TION OF OFFICIAL AGRICULTURAL CHEMISTS

J. A. LeClerc, General Referee on Cereal Products, 1934

Bureau of Chemistry and Soils, U. S. Department of Agriculture,
Washington, D. C.

This year marks the 50th anniversary of the founding of the A. O. A. C.; so far as the study of problems connected with cereal chemistry is concerned this is the 33rd anniversary.

The first referee on Methods of Analysis applicable to cereals and cereal products served during 1901. After that date the appointment of an associate referee on Methods of Analysis relating to cereals and cereal products was made annually. To date the following chemists have served as referees:

Anthony McGill 1901–07, E. F. Ladd 1908–09, W. M. Allen 1910, H. L. White 1911–13, B. R. Jacobs 1914; L. A. Fitz 1915; J. A. Le-Clerc 1916–19, C. H. Bailey 1920–22, C. E. Mangels 1923, R. Hertwig 1924–25, F. C. Blanck 1926–27, J. A. LeClerc 1928–34.

Bureau of Chemistry Bulletin 107 (Official and Provisional Methods of Analysis) was issued in 1908. This bulletin of 272 pages has only a chapter heading " Cereal Foods " with the footnote " in preparation." Marked advancements and developments have taken place relative to methods of analysis applicable to cereals and cereal products since the appointment of the first referee on this subject by the A. O. A. C. as will be noted by observing the section devoted to cereals in the third edition of the Methods of Analysis of the Association of Official Agricultural Chemists. In this edition eighteen closely printed pages are devoted to the analysis of flour, bread, and macaroni products; about 45 methods of analysis are given in more or less detail.

The importance of cereal chemistry is apparent to all students of the subject. Cereals and cereal products play one of the most important rôles in our industrial structure, as about 30% of all food products produced or manufactured in this country are composed wholly or at least in part of cereals and this does not include beer and alcoholic liquors. Prewar some 60,000,000 barrels of beer and approximately 200,000,000 gallons of distilled spirits were produced, requiring in all considerably more than 100,000,000 bushels of barley, corn and rice combined. Furthermore, cereals and cereal products constitute no inconsiderable

part of the average American diet.   The per capita consumption of these foods in this country is approximately as follows:

	Pounds
Wheat flour	171.0
Corn flour, hominy, starch, corn breakfast foods	28.5
Corn sirup, corn sugar	12.2
Polished rice	5.9
Macaroni products	4.5

Mention should also be made of breakfast foods other than from corn, pearl barley, malt sirup, etc.

These foods are sufficient to furnish over one-third of the energy required by the average person.

The problems connected with milling, baking, biscuit and cracker manufacture, brewing and distilling, not to mention the somewhat smaller cereal industries, are such as to command the best trained minds for solution.   The cereal chemist must at all times keep abreast with chemical progress everywhere, for this branch of science is not merely national in scope but worldwide.   Some of the best work in cereal chemistry is being done in many of the European and South American countries and even in Asia and in Australia.

All will admit that much has already been accomplished in the field of cereal chemistry.   There is no doubt also that much remains to be done.   The A. O. A. C. should soon adopt methods for the analysis of brewing materials and improve those for beer and other alcoholic beverages.   Last year attention was called to the desirability of enlarging the scope of the work of the Cereal Section so as to include the study of those materials which enter the manufacture of malt and distilled liquors.   In consideration of this, the A. O. A. C. will this year appoint an Associate Referee to undertake the study of foreign and domestic methods of analysis applicable to cereals and cereal products used in brewing.

Special Papers and Reports of Associate Referees

R. M. Bohn, Director, Technical Institute of the Independent Biscuit Manufacturers' Company, spoke on biscuit and cracker manufacture. He gave a description of the machines and procedures used in the manufacture of the various types of crackers and biscuits, illustrating his talk with slides.   Many of the products of the industry were exhibited and considerable technical information developed regarding the types and characteristics of flour best suited for the manufacture of the various types of biscuit goods.

Associate Referee L. H. Bailey then presented his report on "Ash and Moisture in Flour and Baked Products," suggesting that the present methods for ash and chlorides be supplemented by one for determining

" Salt-free ash," *viz.,* " Deduct from the total Cl in macaroni or similar products the Cl normally present in semolina or flour. Calculate the balance of the Cl to NaCl and subtract this from the total ash of the macaroni."

Chlorides in baked products may be determined in the same manner as chlorides in alimentary pastes. But a salt-free ash of bread and other baked products which may contain besides flour, yeast, yeast food, malt, milk, etc., will not be the same as the ash of the flour from which the baked products were made.

An approximation to the true ash value of the original flour may be obtained in the same way as is sometimes done in the case of macaroni, *viz.,*—determine the P_2O_5 in the ash of the product, multiplying this value by the proper factor to obtain the flour ash. Obviously this method cannot be used with products prepared with phosphorus-containing raw materials other than flour.

In his study of moisture in baked products containing fruit, the Associate Referee found that it was quite impossible to obtain constant weight either at 130° C. or in a vacuum oven at temperature of boiling water, and hence recommended that no special method be adopted as official for moisture in baked products containing fruit.

The report of Associate Referee Rowland J. Clark, on " H-ion Concentration in Flour," was read by Dr. Grewe. This report gave the results from 4 collaborators from which it is evident that real progress is being made toward the establishment of a quick and reasonably accurate method for the colorimetric determination of pH in flour, macaroni and bread.

The report of Associate Referee M. J. Blish, on " The Diastatic Value of Flour," was read by one of the collaborators (L. H. Bailey). Seventeen chemists assisted in this investigation and the results were so satisfactory that the method was advanced to the status of " official " (first action). The method will be given in full in a coming number of the Journal of the Association of Official Agricultural Chemists, (see also J. A. O. A. C. **17**, p. 394, 1933).

The report on " Flour Bleaching Chemicals," was presented by Associate Referee Dorothy B. Scott who stated that progress was being made toward the development of a more satisfactory method than any heretofore in use and recommended that work on this subject be continued.

B. R. Jacobs read a special paper on " Macaroni Products," in which he gave a short history of the development of the industry in this country. As the result of the passage by Congress of the N. I. R. A. and the A. A. A. the macaroni industry proposed a code of fair competition which has been approved. The chief features of this code, as described

by Mr. Jacobs, are: prohibition of false advertizing; a statement of the net weight of contents; the inclusion in the code of the U. S. D. A. standards for egg-macaroni products; prohibition of the use of any colored wrappers for deceptive purposes; prohibiting the sale of products below cost and the establishment of formulas for determining costs. The speaker felt that as the result of the code the quality of macaroni now on sale was higher and that the industry was better able now to fight against and prevent misbranding, adulteration and unfair competition.

The method for the determination of "Crude fiber" in baked products (not containing fruit) was reported by Associate Referee Ruth G. Capen and made "official" (final action).

Associate Referee V. E. Munsey reported on a new method for determining milk solids in milk bread. This depends upon the determination of the total fat and "fat number" in conjunction with that of non-fat milk solids.

The report of C. G. Harrel, Associate Referee, on Standardization of Viscosity Determination, was read by Dr. F. L. Dunlap. This is a modification of the method first proposed by E. G. Bayfield (Cereal Chemistry X, 494) and was adopted as a "tentative" method.

Clinton L. Brooke, Associate Referee on the "Determination of Ergot in Wheat and Rye Flours" gave (through B. D. Ingels) a summary of the outstanding work found in the literature, which will help to clear the way for development of a definite method.

Associate Referee H. K. Parker's reports on "Color in Flour," was a noteworthy contribution on this subject, which for many years has had little attention given to it by this Association. A real step forward has been taken. The Associate Referee discussed the two principle lines of attack generally followed for the determination and evaluation of color in flour, viz., the extraction methods and the reflectence methods, laying particular stress upon the latter, as they are better suited for the study of color as a whole. It was proposed by means of the spinning disk reflectence to study the correlation between the color of flour and water doughs and bread crumb.

Associate Referee A. K. Balls presented a paper on "Proteolytic Enzymes in Flour," which was read by W. S. Hale. This paper will be published in full in the Proceedings.

Dr. R. Harcourt, President of the A. O. A. C. not only gave the "Annual Address of the President," which was delivered before the whole Association (this will be published in full in the Proceedings), but he also took time to express a few words of greeting to his colleagues in cereal chemistry in the Cereal Section. This is the first time since 1914 (when ex-Senator Ladd served as President) that the cereal

chemists have been honored by having one of its own group made president of the association.

For the first time in the history of the Association, a special paper relating to malting and brewing problems was featured. The paper " Modern Trends in Malting and Brewing Chemistry," by F. P. Siebel, and read by Miss Elsie Singruen, elicited considerable interest. Just previous to the reading of this paper a moving picture was shown depicting the various steps in brewing.

The last paper was by Jos. W. E. Harrison on " Soybean Flour in Smoked Meat Products." The conclusions drawn were, (1) the presence of an urea-splitting enzyme in smoked meat products points towards the inclusion of soy flour; (2) a negative test does not exclude the use of soy flour.

Altogether this was the most successful and well-attended meeting ever held by the Cereal Chemistry Section of the A. O. A. C.

ERRATUM

STUDIES ON THE VITALITY OF WHEAT. III. VITALITY AND THE ACTION OF HEAT ON WHEAT SEEDS, R. Whymper and A. Bradley. Vol. XI, pages 632, 633 and 634, Table III, box heading third column, for " Moisture normal unheated samples " read " Moisture in samples."